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(54) Title: TOPOISOMERASE INHIBITORS

(57) Abstract

The invention provides a novel series of tetracyclic quinoline and quinoxaline carboxamides, *bis* compounds in which two of the tetracyclic compounds are joined by a linker, and pharmaceutically–acceptable salts and N–oxides thereof, which have the ability to inhibit topoisomerase activity. The compounds are active *in vitro* and *in vivo* against tumour cells. Methods of synthesis and pharmaceutical compositions are also claimed.

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TOPOISOMERASE INHIBITORS

This invention relates to compounds with topoisomerase-inhibitory activity, to pharmaceutical compositions comprising these compounds, and to the use of the compounds in the treatment of cancers.

BACKGROUND OF THE INVENTION

Despite the great advances in chemotherapy of cancer which have been made over the last decades, there are still a number of types of cancers for which the response rates are poor, and the agents which are available to treat these conditions have very significant toxic side-effects.

In particular, solid tumours such as cancers of the colon, breast and lung are extremely common, and although each can be treated with presently-available cytotoxic agents, response rates are poor, relapse is common, and the five-year survival rate is poor. Furthermore, the incidence of melanoma is very high, particularly among fair-skinned people, and is increasing; only 25-30% of patients with disseminated melanoma show a response to treatment, and remission is sustained in only 5-10% of patients (Evans *et al*, 1990).

Since the initial discovery of the enzymes DNA topoisomerase I (topo I) and DNA topoisomerase II (topo II; DNA gyrase), intensive effort has been directed at identifying inhibitors of these enzymes and evaluating their activity as potential anti-cancer agents. In particular, inhibitors of topo II have been viewed as attractive targets for drug development. Topo II has the ability to break both strands of the DNA double helix simultaneously in order to catalyse the passage of one DNA molecule through another. Because DNA topoisomerases are essential for many aspects of cell multiplication, they are potentially very useful as anti-tumour agents.

Inhibitors of topoisomerase are usually compounds which have the ability to intercalate into the DNA double helix, and in particular many of these agents bind to the minor groove of the double helix. These agents include synthetic intercalating drugs, such as the aminoacridines, antibiotics such as anthracylines, including doxorubicin, and plant-derived agents such as the ellipticines, epipodophyllotoxins, and camptothecins. In addition, some inhibitors of topoisomerase do not intercalate into the DNA, and these include etoposide, teniposide, and compounds such as merbarone and aclarubicine which do not form cleavable complexes with DNA. A number of compounds are in advanced clinical development, and several are in clinical use. The subject has been reviewed (see for example Chen & Liu, 1994; chapter on "Oncolytic Drugs" in "This Year's Drug News", Prous Publishers, 1995).

Following the clinical success of the DNA-intercalating topo II inhibitors doxorubicin, mitoxantrone and their analogues as anticancer drugs, a great deal of work has been devoted towards other classes of compounds with similar overall topology, *ie*. polycyclic chromophores bearing a flexible cationic side chain, as topo II inhibitors. Among the more successful examples are the benzoisoquinolinediones such as mitonafide (**1**) (Rosell *et al*, 1992) the anthrapyrazoles such as losoxantrone (**2**) (Judson, 1992) and the phenazine-1-carboxamides (*eg*. **3**) (Rewcastle *et al*, 1987). More recently, interest has focused on compounds with the ability to inhibit both topo I and topo II enzymes. Examples of such "mixed" inhibitors which show broad-spectrum activity against solid tumours and are in clinical trial include the acridine-4-carboxamide N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) (**4**) (Atwell *et al*, 1987; Baguley *et al*, 1995; also U.S. Patent No. 4,590,277 and International Patent Application No. WO 93/24096), the imidazoacridanone (**5**) (Dziegielewski and Konopa, 1996), and various tetracyclic chromophores (*eg*. **6**) (Utsugi *et al*, 1996). Formulae of these compounds are presented in Figure 1.

DACA has been found to have a different *in vitro* cytotoxicity profile to amsacrine and etoposide, and in particular is active against cell lines which show both P-glycoprotein-mediated (transport) drug resistance and "atypical" or "altered" multidrug resistance. Thus, especially when used in combination with drugs which act via a different cytotoxic mechanism, DACA and related compounds have the potential to be able to circumvent multidrug resistance (WO 93/24096).

In testing drugs for anti-cancer activity, a panel of mouse tumours is commonly used. These include transplantable leukaemias, the Lewis lung adenocarcinoma and colon **38** adenocarcinoma. Xenografts of human tumours in immunodeficient nude mice are also increasingly widely used, for example human melanoma xenografts (Taetle *et al*, 1987). Lewis lung adenocarcinoma is regarded as a good model for human small-cell lung cancer (Zacharski, 1986), and colon **38** tumour is regarded as a useful model for human colon cancer (Corbett *et al*, 1975).

From the review articles referred to above it can be seen that compounds which have activity against topo II show a wide variety of structures. Although there are some families of compounds, within a given family a wide variety of substituents on the central ring structure(s) is possible.

Some of the known topo II inhibitors which are being developed as anti-cancer agents are fused tetracyclic systems, for example the ellipticines and batracyclin. Both of these compounds comprise two 6-membered rings linked to a third 6-membered ring via a 5-membered ring. Methyl-substituted derivatives of benzopsoralen and benzoangelicin have been synthesised, and one of these compounds, 4-hydroxymethyl benzopsoralen, has been shown not only to act as a photoreactive DNA synthesis inhibitor activated by ultraviolet-A, but also to have topo II inhibitory activity (Guitto *et al*, 1994). Substituted tetracyclic fused quinoline derivatives are disclosed in

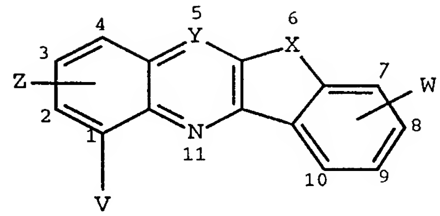
International Patent Publication No. WO 92/21661 and U.S. Patent No. 5,223,506, both by Glaxo, Inc. International Patent Publication No. WO 94/24135 by Taisho Pharmaceutical Co., Ltd. discloses indolo[2,3-b]-quinoxaline derivatives. One of the present inventors has described synthesis of certain 11*H*-indeno[1,2-*b*]quinoxalin-11-ones (Deady *et al*, 1993). However, none of these documents discloses a carboxamide or substituted carboxamide side chain *peri* to an aromatic nitrogen). Thus none of these publications discloses or suggests the compounds of the present invention.

Because the relationships between structure and the ability to inhibit topoisomerases are still not well defined, and because of the potential utility of such compounds, further classes of such agents are needed. Within the broad subclass of polycyclic heterocyclic carboxamides, the nature and positioning of the carboxamide side chain has been shown to be critical, with attachment to a terminal ring *peri* to an electron-withdrawing atom in the central ring being required for biological activity, and the acridine-4-carboxamides and phenazine-1-carboxamides being the most biologically active of the series (Palmer *et al*, 1988; Chen *et al*, 1994).

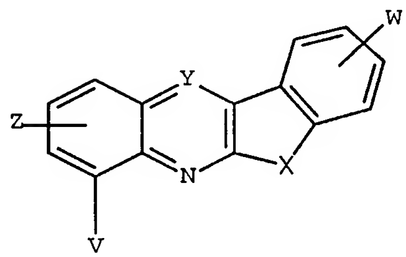
We have now synthesised and evaluated a novel series of tetracyclic quinoline- and quinoxaline-carboxamides, and have found that representative examples of these compounds show good activity against model tumour systems.

SUMMARY OF THE INVENTION

According to a first aspect, the invention provides a compound of general formula I or general formula II



I



II

in which positional numbering, where mentioned, refers to the arbitary system illustrated for formula I above, and

in which V is $C(=U)NR(CH_2)_nR^1$, where U is O or S, R is hydrogen or a C_{1-4} alkyl group which is optionally substituted with one or more OH or NH_2 groups, and R^1 is $C(=NH)NH_2$, $NHC(=NH)NH_2$ or NR^2R^3, where each of R^2 and R^3 is independently hydrogen or a C_{1-4} alkyl group which is optionally substituted with one or more OH or NH_2 groups, and n is an integer from 1 to 6;

Y is CH, N or C-V

X is CH_2, CH-C_{1-4} alkyl, CO, O, S, SO, SO_2, N-C_{1-4} alkyl or NH;

Z is H, F, Cl, Br, I, OH, NR^2R^3, nitro, cyano, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy 2,3- or 3,4-methylenedioxy, or 3,4-ethylenedioxy; and

W is H, F, Cl, Br, I, OH, NR^2R^3, nitro, amino, cyano, benzo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, 7,8- 8,9- or 9,10-methylenedioxy or ethylenedioxy,

or a pharmaceutically-acceptable salt or N-oxide thereof.

When W is benzo, this may be linked 7,8; 8,9; 9,10; or 6,6a,7.

When X is CH_2 or NH, a hydrogen may optionally be substituted with a C_{1-4} alkyl group.

Preferably X is NH, CO, CH_2, O or S; Y is CH, N or C-CONH$(CH_2)_2$N$(CH_3)_2$; Z is H, methoxy or Cl; W is H, methoxy, methyl, Cl, hydroxy or benzo; and n is 2.

More preferably the compound is a compound of general formula I, and X is CO, Y is CH, and Z is H.

In particularly preferred embodiments the compound is N-[2-(dimethylamino)ethyl]-11-oxo-11*H*-indeno[1,2-*b*]-quinoline-6-carboxamide (**12a**) or N-[2-(dimethylamino)-ethyl]-11-oxo-4-methyl-11H-indeno[1,2-b]quinoline-6-carboxamide (**12g**), or a pharmaceutically acceptance salt or N-oxide thereof.

It is known that many topoisomerase inhibitors are *bis* compounds, such as *bis*-imidazoacridones, *bis*-triazenoacridones, and the compounds DMP840 and LU79553, which are respectively in Phase II clinical trial and about to commence in Phase I clinical trial. Consequently in a preferred aspect the invention provides compounds in which two units of general formula I or general formula II respectively, which may be the same or different, are linked via a linker group. It will be clearly understood that these *bis* compounds of the invention include the salts and N-oxide forms of compounds of general formula I or general formula II.

When the linkage is through V, the group NR$(CH_2)_n$R^1 is replaced in each subunit of the *bis* compound by a linker group selected from the group consisting of:

-NH$(CH_2)_3$NH$(CH_2)_4$NH-

-NH$(CH_2)_3$NH$(CH_2)_3$NH$(CH_2)_3$NH-

-NH$(CH_2)_2$NH$(CH_2)_2$NH-

-NH$(CH_2)_3$-NMe-$(CH_2)_3$NH-

-NH$(CH_2)_2$NH$(CH_2)_2$NH$(CH_2)_2$NH-

-NH$(CH_2)_2$NH$(CH_2)_3$NH$(CH_2)_2$NH-

-NH$(CH_2)_2$NMe$(CH_2)_2$NMe$(CH_2)_2$NH-

$-NH(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2NH-$
-N,N'-Bis(2-aminoethyl)piperazine-
N,N'-Bis(3-aminopropyl)piperazine,
where Me represents methyl.

In particularly preferred embodiments, the compounds are N,N'-[[(2-Aminoethyl)imino]di-3,1-propanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**74**), N,N'-[[(2-aminoethyl)methylimino]di-3,1-propanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**76**), N,N'-[[(2-aminoethyl)imino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**78**) and N,N'-[[(2-aminoethyl)methylimino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**79**), or pharmaceutically-acceptable salts or N-oxides thereof.

The linker may be symmetrical or non-symmetrical. Other linker groups contemplated by the invention are generally of the form $-NH(CH_2)_mNH(CH_2)_nNH-$ or $-NH(CH_2)_mNAlkyl(CH_2)_nNH$ or $-NH(CH_2)_mNH(CH_2)_nNH(CH_2)_oNH-$ or $-NH(CH_2)_mNAlkyl(CH_2)_nNAlkyl(CH_2)_oNH-$, where m, n and o are integers from 2 to 6, or linkers of the type disclosed in International Patent Application No. WO 96/25400 by The Du Pont Merck Pharmaceutical Company, the entire disclosure of which is incorporated by this cross-reference.

When the linkage is through X, the H of CH_2 or NH is replaced in each subunit by a link via $-(CH_2)_m$, where m is an integer from 1 to 12; O of C=O is replaced in each subunit by a bis-olefinic link via $=CH(CH_2)_n-CH=$; or =O of C=O is replaced in each subunit by a bis-oxime link via $=N-O-(CH_2)_p-O-N=$, where p is an integer from 1 to 8.

Methods for preparation of *bis* compounds from their subunits are known in the art.

It will be clearly understood that methods of synthesis of compounds of the invention also form part of the invention. Certain intermediate compounds described herein are novel, in particular all precursor acids to the amides of the invention (except 11-oxo-11H-indeno[1,2-

b]quinoxaline-6-carboxylic acid (Deady *et al*, 1993)), and these acids also form part of the invention.

In a third aspect, the invention provides a pharmaceutical composition comprising a compound of general formula I, general formula II or a *bis* compound as described above, together with a pharmaceutically-acceptable carrier.

In a fourth aspect, the invention provides a method of treatment of a neoplastic condition, comprising the step of administering an anti-tumour effective dose of a compound of the invention to a mammal in need of such treatment. The compound of the invention may be administered simultaneously or sequentially with one or more other anti-neoplastic agents, including but not limited to anti-mitotic agents *eg*. taxol, anti-metabolites *eg*. 5FU, hormonal regulators *eg*. tamoxifen, DNA reactive agents *eg*. cisplatin, or biological agents *eg*. IL-2 or antibodies. The compound of the invention may also be used in combination with agents which relieve symptoms caused by drug treatment *eg*. GM-CSF, or anti-emetics. Preferably the second agent is a cytotoxic drug which is not a topo II inhibitor; more preferably the second anti-neoplastic agent is a DNA-binding anti-cancer agent.

The mammal may be a human, or may be a domestic or companion mammal such as a horse, bovine, sheep, dog or cat, or a non-human primate.

Optionally the compound of the invention is administered in a divided dose schedule, such that there are at least two administrations in total in the schedule. Administrations are given preferably at least every two hours for up to four hours or longer; for example the compound may be administered every hour or every half hour. In one preferred embodiment, the divided-dose regimen comprises a second administration of the compound of the invention after an interval from the first administration sufficiently long that the level of active compound in the blood has decreased to approximately from 5-30% of the

maximum plasma level reached after the first administration, so as to maintain an effective content of active agent in the blood. Optionally one or more subsequent administrations may be given at a corresponding interval from each preceding administration, preferably when the plasma level has decreased to approximately from 10-50% of the immediately-preceding maximum.

Preferably a second DNA-binding anti-cancer therapeutic agent is used in conjunction with administration of the compound of the invention in order to reduce toxicity to the recipient of either or both of the compound of the invention or the other anti-cancer agent; the compound of the invention and the other agent may be administered together or sequentially.

It is contemplated that compounds of the invention may also be administered in the form of tumour-activated prodrugs, in which the active agent is linked to a "trigger" domain; such compounds may for example be designed to be activated by local hypoxia within a tumour mass. See for example Denny, 1996; McFadyen *et al*, 1996.

The compounds of the invention may be administered by any suitable route, for example orally, buccally, topically or parenterally, for example by intravenous, sub-cutaneous, intramuscular, intra-peritoneal, or intratumoural injection. The dose and route of administration will depend on the condition to be treated, and will be at the discretion of the attending physician or veterinarian. It is contemplated that each administration will supply between 0.1 and 500 mg, preferably 1 to 200, more preferably 1 to 50 mg of active compound.

The compounds of the invention are suitably presented in unit dosage form, and the person skilled in the art will be aware of suitable carriers and formulations, for example by reference to textbooks such as Remington's Pharmaceutical Sciences, 10th Edition.

The compounds of the invention may be used in the treatment of leukaemias, lymphomas, sarcomas, and brain

tumours, and for cancers of the lung, breast, ovary, testes, and colon.

Brief Description of the Drawings

Figure 1 shows the formulae of prior art compounds **1** to **6** referred to herein;

Figure 2 shows reaction Schemes 1a, 1b and 1c for synthesis of precursor acids of the quinoline-based compounds of the invention;

Figure 3 shows reaction Scheme 2 for synthesis of an isomeric quinoline acid **47**;

Figure 4 illustrates reaction Schemes 3a, which provides a common pathway for synthesis of the quinoxaline-based acids, and 3b, which illustrates the synthesis of a representative example of a quinoxaline-based acid **49**;

Figure 5 shows the structures of intermediates relevant to the synthesis of quinoxaline-based acids and the structure of the imidazolide, **68**, derived from compound **42a**;

Figure 6 shows reaction scheme 4a for preparation of most amide compounds of Table 1 from the corresponding acids, and a modified reaction scheme 4b for synthesis of amide compounds from quinoxaline-based acids in which the 5-membered ring comprises an NH group; and

Figure 7 shows the growth of colon 38 tumours implanted subcutaneously in C57Bl/6 mice, which were treated with compound **12a** at a dose of 60 mg/kg (■) or 90 mg/kg (Δ), compared to untreated mice (●) as described in Example 10.

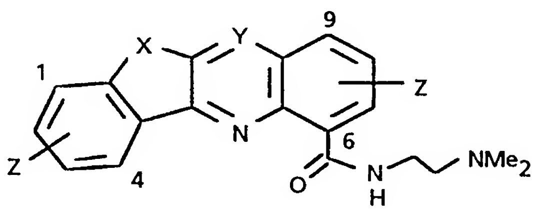
Figure 8 shows the growth of colon 38 tumours implanted subcutaneously in C_{57}Bl/6 mice, which were treated with compound **10** at a dose of 20 mg/kg as described in Example 10.

DETAILED DESCRIPTION OF THE INVENTION

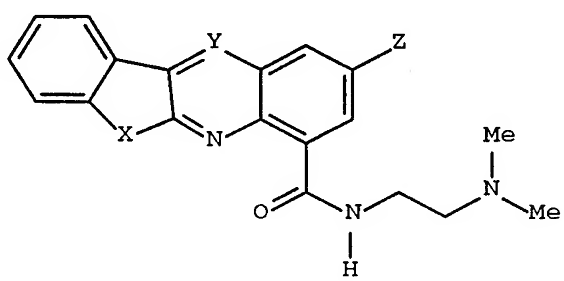
The invention will now be described in detail by way of reference only to the following non-limiting examples, and to the drawings, in which:

The structure of representative compounds of the invention is summarised in Table 1. It will be evident that form A represents a sub-set of compounds of general formula I and form B represents a sub-set of compounds of general formula II respectively.

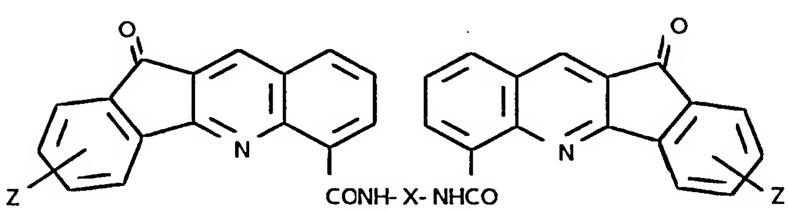
Table 1



A



B



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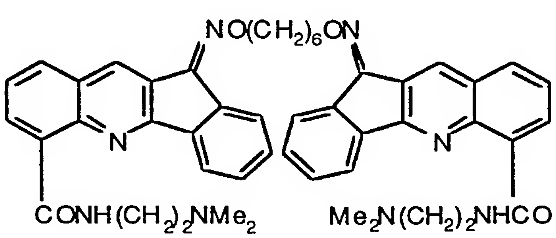

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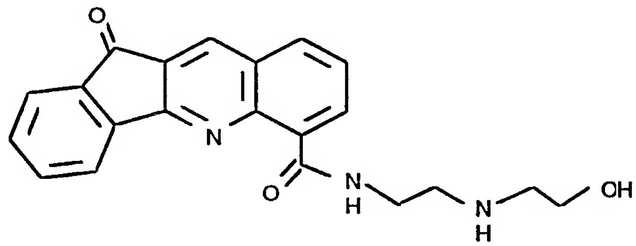
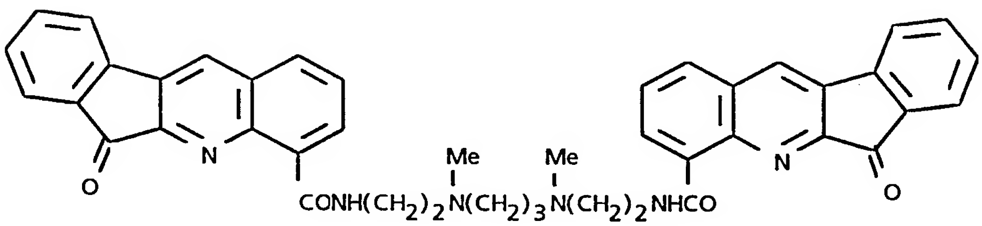
Table 1 (cont.)



E



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Representative Compounds of the Invention

Number	Form	X	Y	Z
7	A	NH	CH	H
8	A	O	CH	H
9	A	S	CH	H
10a	A	S	C-R^e	H
10b	A	CO	C-R^e	H
10c	A	CO	C-R^e	4-Me
11	A	CH_2	CH	H
12a	A	CO	CH	H
12b	A	CO	CH	1-OMe
12c	A	CO	CH	2-OMe
12d	A	CO	CH	3-OMe
12e	A	CO	CH	4-OMe
12f	A	CO	CH	3-Me
12h	A	CO	CH	2-Cl

12i	A	CO	CH	4-Cl
12g	A	CO	CH	4-Me
12j	A	CO	CH	2,3-$(OMe)_2$
12k	A	CO	CH	1,11a,11-benzo
12m	A	CO	CH	8-OMe
12n	A	CO	CH	8-Cl
12o	A	CO	CH	3-OH
13	A	SO_2	CH	H
14	A	NH	N	8-Cl
15	A	O	N	8-Cl
16	A	CH_2	N	H
17	A	CO	N	H
18	A	S	N	H
19	B	CO	CH	H
20	B	NH	N	H
21	B	NH	N	8-Cl
22	B	O	N	H
23	B	O	N	8-Cl
69	C	$(CH_2)_2NH(CH_2)_2$	na	H
70	C	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	na	H
71	C	$(CH_2)_3Nme(CH_2)_3$	na	H
72	C	$(CH_2)_3$-1,4-piperazinediyl-$(CH_2)_3$	na	H
73	D	na	na	na
74	C	$(CH_2)_2NH(CH_2)_3NH(CH_2)_2$	na	H
75	C	$(CH_2)_2NMe(CH_2)_2NMe(CH_2)_2$	na	H
76	C	$(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2$	na	H
77	C	$(CH_2)_3NH(CH_2)_4$		H
78	C	$(CH_2)_2NH(CH_2)_3NH(CH_2)_2$		4-Me
79	C	$(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2$		4-Me
80	C	$(CH_2)_3NH(CH_2)_3NH(CH_2)_3$		H
81	F			
82	E			

[e]R = $CONH(CH_2)_2NMe_2$

na = not applicabale

<u>Elemental Analyses for New Compounds</u>

8	Calc. for $C_{20}H_{19}N_3O_2$: C, 72.0; H, 5.7; N, 12.6.
	Found: C, 72.1; H, 5.7; N, 12.8.
10a	Calc. for $C_{25}H_{29}N_5O_2S$: C, 64.7; H, 6.3; N, 15.1.
	Found: C, 64.5, H, 6.2; N, 15.0.
10b	Calc. for $C_{26}H_{29}N_5O_3.H_2O$: C, 65.4; H, 6.4; N, 14.7.
	Found: C, 65.7: H, 6.8: N, 14.8
10c	Calc for $C_{27}H_{31}N_5O_3.H_2O$: C, 66.0; H, 6.8; N, 14.3.
	Found: C, 66.3; H, 6.1; N, 14.3.
11	Calc. for $C_{21}H_{21}N_3O$: C, 76.0; H, 6.4; N, 12.7.
	Found: C, 75.8; H, 6.3; N, 12.6.
12a	Calc. for $C_{21}H_{19}N_3O_2$: C, 73.0; H, 5.5; N, 12.2.
	Found: C, 72.9; H, 5.4; N, 12.3.
12c	Calc. for $C_{22}H_{21}N_3O_3$: C, 70.4; H, 5.6; N, 11.2.
	Found: C, 70.3; H, 5.4; N, 11.1.
12d	Calc. for $C_{22}H_{21}N_3O_3$: C, 70.4; H, 5.6; N, 11.2.
	Found: C, 70.3; H, 5.3; N, 11.1.
12e	Calc. for $C_{22}H_{21}N_3O_3.H_2O$: C, 67.4; H, 6.0; N, 10.85.
	Found: C, 67.2; H, 5.9; N, 10.7.
12f	Calc. for $C_{22}H_{21}N_3O_2$: C, 73.5; H, 5.9; N, 11.7.
	Found: C, 73.2; H, 6.1; N, 11.7.
12g	Calc. for $C_{22}H_{21}N_3O_2.0.5H_2O$: C, 71.7; H, 6.0; N,11.4.
	Found: C, 71.8; H, 5.7; N, 11.6.
12h	Calc. for $C_{21}H_{18}ClN_3O_2$: C, 66.5; H, 4.8; N, 11.1.
	Found: C, 66.4; H, 4.6; N, 11.2.
12i	Calc. for $C_{21}H_{18}N_3O_2Cl.0.5H_2O$: C, 64.9; H, 4.9; N, 10.8.
	Found: C, 65.3; H, 4.8; N, 10.8.
12j	Calc. for $C_{23}H_{23}N_3O_4$: C, 68.1; H, 5.7; N, 10.4.
	Found: C, 67.8: H, 5.7: N, 10.4.
12k	Calc. for $C_{24}H_{21}N_3O$: C, 78.4; H, 5.8; N, 11.4.
	Found: C, 78.4; H, 6.0; N, 11.6.
12m	Calc. for $C_{22}H_{21}N_3O_3.0.5H_2O$: C, 68.7; H, 5.8; N, 10.9.
	Found: C, 68.6; H, 5.5; N, 10.9.

12n	Calc. for $C_{21}H_{18}ClN_3O_2$: C, 66.4; H, 4.8; N, 11.1. Found: C, 66.3: H, 4.9: N, 11.2.
13	Calc. for $C_{20}H_{18}N_3O_3S$: C, 63.1; H, 4.8; N, 11.0. Found: C, 63.2; H, 4.8; N, 10.9.
14/21	Calc. for $C_{19}H_{18}ClN_5O.HClO_4.0.5H_2O$: C, 47.8; H, 4.2; N, 14.7. Found: C, 47.9; H, 4.2; N, 14.5.
16	Calc. for $C_{20}H_{20}N_4O$: C, 72.3; H, 6.1; N, 16.9. Found: C, 71.8; H, 5.7; N, 16.9.
17	Calc. for $C_{20}H_{18}N_4O_2$: C,69.4; H, 5.2; N, 16.2. Found: C, 69.3; H, 5.2; N, 16.1.
18	Calc. for $C_{19}H_{18}N_4OS$: C, 65.4; H, 5.2; N, 16.0. Found: C, 65.4, H, 5.1; N, 16.3.
19	Calc. for $C_{21}H_{19}N_3O_2$: C, 73.0; H, 5.5; N, 12.2. Found: C, 72.8; H, 5.6; N, 11.9.
15/23	Calc. for $C_{19}H_{17}ClN_4O_2$: C, 61.9; H, 4.6; N, 15.2. Found: C, 61.4; H, 4.4; N, 15.0.
69	Calc. for $C_{38}H_{27}N_5O_4.H_2O$: C, 71.8; H, 4.6; N, 11.0. Found: C, 72.0: H, 4.5: N, 11.2.
70	Calc. for $C_{40}H_{32}N_6O_4.2.5H_2O$: C, 68.1; H, 5.3; N, 11.9. Found: C, 68.1: H, 5.3: N, 12.1.
72	Calc. for $C_{44}H_{38}N_6O_4.H_2O$ C, 72.1; H, 5.5; N, 11.4. Found: C, 72.2: H, 5.3: N, 11.2.
73	Calc. for $C_{48}H_{50}N_8O_4.2HClO_4.4H_20$ C, 53.6; H, 5.6; N, 10.4. Found: C, 54.0: H, 5.3: N, 10.1.
74	Calc. for $C_{41}H_{34}N_6O_4.H_2O$: C, 71.1; H, 5.2; N, 12.4. Found: C, 71.2: H, 5.1: N, 12.1
75	Calc. for $C_{42}H_{36}N_6O_4.0.5H_2O$: C, 72.3; H, 5.3; N, 12.0. Found: C, 72.3: H, 5.3: N, 12.3.
78	Calc for $C_{43}H_{38}N_6O_4.3H_2O$: C, 68.2; H, 5.9; N, 11.1. Found: C, 68.6; H, 5.9; N, 11.0.
82	Calc. for $C_{21}H_{19}N_3O_3$: C, 69.8; H, 5.3; N, 11.6. Found: C, 69.2: H, 5.5: N, 11.7.

Quinoline-based Compounds

The basic strategy for synthesis of the quinoline based compounds (**7-13**, **19**, **69-76**) of Table 1 involved an adaptation of the Pfitzinger synthesis (Figure 2), in which a 7-substituted isatin (**24** or **25**) was reacted with a ketone to give the tetracycles **30-33** (Scheme 1a), **35** (Scheme 1b). Most work utilized isatins **24**, with the final acid function already in place. These were prepared by a modification of a literature method (Waldmann, 1937), and reactions with **26-29** gave **30-33**. Alternatively 7-methylisatin (**25**) (Chen and Deady, 1993) may be used as a starting material, wherein the methyl group can be oxidized to the desired carboxylic acid after the tetracylic system has been constructed. An example of this sequence is the reaction of **25** with **28** to give the tetracycle **35**. Many variations on base catalysis have been employed in the Pfitzinger reaction (Jones, 1977). A number were tried in the present work, and 10% aqueous sodium hydroxide at 90-100°C (Noelting and Herzbaum, 1911), in some cases with added ethanol to aid solubility, was found satisfactory for all except one reaction. For **26**, the literature procedure (Holt and Petrow, 1947) for a related compound (20% potassium hydroxide at room temperature for 10 days in the dark) proved superior, although the yield of **30** was only 21% and was accompanied by much indigo formation. Workup of the reactions was quite individual with respect to the species (salt or free acid) which separated at particular acidities, as set out in detail in the examples. The high melting acids were generally difficult to purify completely, and microanalytical characterization was confined to the final amides.

Thermal decarboxylation of the initial condensation products was then required to give the acids **38-43**. In most cases where C=O as X was desired, oxidation of the CH_2 precursor **33** with $KMnO_4$ under carefully controlled conditions was carried out prior to decarboxylation. Where a methoxy group was present in ring A, **33m**, this and

various chromium oxidants failed, but nickel peroxide (Nakagawa et al, 1962) under mild conditions gave **34m** in 65% yield. Otherwise decarboxylation of the appropriate **33** preceded oxidation with $Na_2Cr_2O_7$ in hot acetic acid. Attention to detail was required in the decarboxylation process in order to obtain good yields. Decarboxylation was carried out by heating the solid diacids: (a), at atmospheric pressure approximately to their melting points while being observed under mild magnification, and heating was discontinued after a few minutes when the obvious reaction had ceased, or (b), at reduced pressure with a small Bunsen flame, when the product mono acid sublimed onto a cold-finger.

Preparation of the 4-methoxysubstituted compound had to be modified, as decarboxylation of a 4-OMe diacid (whether X = CH_2 or CO) was accompanied by some demethylation which complicated purification. Aluminium chloride demethylation of the oxidized Pfitzinger product **34e**, was therefore first carried out, followed by clean decarboxylation of **341** to **421**, and then reaction with MeI and silver oxide methylated both acid and phenol OH groups (Scheme 1c). The product, **36**, when heated with the appropriate amine, gave the required amide **12e** directly. The same oxidation/demethylation/decarboxylation sequence from the 3-methoxy Pfitzinger product **33d** was used to prepare the 3-hydroxy acid **42o** (Scheme 1c).

Oxidation of the methyl substituent in **37** with CrO_3/H_2SO_4 was accompanied by oxidation in the 5-membered ring to give the required monoacid **43** (Scheme 1b).

There is no general method of synthesis of the isomeric quinoline acids in which the orientation of the 5-membered ring is reversed. One member of this series was prepared by the same reaction sequence as above, as shown in Scheme 2 (Figure 3). Isatin-7-carboxylic acid (24a) was reacted with 2-indanone (**44**) to give **45**, which underwent the standard oxidation/decarboxylation sequence to give **47**.

Quinoxaline-based Compounds

Derivatives of systems in which a 5-membered ring containing O, S or NH is inserted into the phenazine system, to give compounds of Form A and Form B, have been prepared.

The requirement for the carboxyl substituent in the position shown introduced some complexity into the synthesis. Furthermore, when the diamine is unsymmetrical there is the task of assigning any product to the isomeric Form A or Form B series. This is an extension of a longstanding problem in quinoxaline chemistry. In the tetracyclic compounds, previous examples have either used a symmetrical diamine or have left the structures unresolved. We have synthesised all three hetero series by a common pathway, which is shown in Scheme 3a (Figure 4a), and have developed methods for establishing individual structures as Form A or Form B.

Synthesis of the new quinoxaline-based acids was by condensation of 2,3-diaminobenzoic acid (**48**), or the 5-chloro analogue **52**, with the appropriate α,β-dicarbonyl compound (see Scheme 3b, shown in Figure 4, for a representative example). Details of the synthesis and assignment of the isomeric acids of this series are reported below, and the structures of intermediates in these syntheses are shown in Figure 5.

Preparation of Amides

The amides of Table 1 were prepared from the corresponding acids by various methods. A number were prepared by a mixed anhydride method (Chen *et al*, 1994), using isobutyl chloroformate as the initial reagent, followed by reaction with *N,N*-dimethylethylene-diamine, as shown in Scheme 4a (Figure 6). Others were prepared by reaction of the acid with 1,1'-carbonylimidazole, and the intermediate imidazolide (an example is structure **68** in Figure 5) was then reacted with the appropriate amine. Yet

others were prepared by generation of an intermediate carbonyl chloride.

The isomeric structures of the quinoxaline-based amides (**14-18**, **20-23**) follow from those determined for the acids. For those acids containing an NH group in the 5-membered ring, some reaction also occurred at this nitrogen. The quinoline-based compound **14** was initially formed by way of the intermediate carbonyl chloride. However, this method gave low yields of impure compounds with the quinoxaline-based analogues, and the mixed anhydride method was modified, as shown in Scheme 4b (Figure 6). For example, reaction of **50** with two equivalents of acylating agent gave the amide/carbamate (**51**), by reaction of both the CO_2H and NH with the isobutyl chloroformate, followed by selective reaction of the anhydride with the amine. Selective hydrolysis of the carbamate group of **51** with mild base then gave **20**. The isomeric amide mixture **14/21** was prepared similarly.

Most of the polyamine linkers required for preparation of bisamides were commercially available. *N,N'*-Bis(2-aminoethyl)-*N,N'*-dimethyl-1,2-ethane- (and 1,3-propane) diamines, though both are known (Braña, Castellano *et al*, 1997) (Braña, M.F.; Pérez de Vega *et al*, 1997) were prepared by a common procedure, slightly different to the existing literature. *N,N'*-dimethylethane- (and propane-) diamines were reacted with chloroacetonitrile, then with borane by a literature procedure for nitrile reduction (Brown and Subba Rao, 1960).

Bisamides were also prepared by both mixed anhydride and imidazolide methods. Examples **71** and **72** were prepared by the former method. The imidazolide method was found to be a more convenient general one and bisamides **69**, **70**, **74-77** and **80** were prepared in this way from imidazolide **68**, and **78**, **79**, **81** from the imidazolides of the corresponding acids. The bisoxime **73** was prepared from

amide **12a** and O,O'-1,6-hexanediylbishydroxylamine in acid conditions.

Experimental Methods

In the following examples, analyses indicated by symbols of the elements were within ± 0.4% of the theoretical.

N.m.r. spectra were recorded on Brüker AM-300 (300.13 and 75.47 Mhz for ^1H and ^{13}C, respectively) and Brüker DRX-400 (400.13 and 100.62 Mhz) spectrometers, in $(CD_3)_2SO$ unless otherwise stated. The electrospray mass spectra were obtained on a VG Bio-Q triple quadruple mass spectrometer using a water/methanol/acetic acid (50:50:1) mobile phase.

In the listings, proton counts for aromatic protons, which have not been assigned, are given only for unresolved multiplets; the other aromatic signals are single proton doublets and triplets with J = 6-8 Hz, except the pyrido ring proton, a singlet. In addition to the peaks listed, all monocarboxamides except **82** had a common pattern for the side chain: δ 2.4 (s, 6 H, $N(CH_3)_2$), 2.7 (t, J = 6 Hz, 2 H, CH_2N), 3.75 (q, J = 6 Hz, 2 H, $NHCH_2$).

The (^{13}C-^1H) HETCOR experiment was performed using the pulse sequence described by Bax and Morris, 1981. The refocusing delay was optimized to 160 Hz (3.0 ms). The spectrum was acquired as 512 x 128 data points, zero filled and subjected to both Fourier transforms to afford the 1024 x 1024 point data matrix. The number of transients per t_1 increment was 128. Spectral widths were 4761 Hz in F_1 (^1H) and 14705 Hz in F_2 (^{13}C). The 90° pulse widths were 14.0 μs (^1H) and 13.5 μs (^{13}C).

The long-range proton detected (three bond) (^1H-^{13}C) heteronuclear multiple bond correlation (HMBC) experiment used the pulse sequence described by Bax and Summers, 1986. The low pass J-filter portion of the experiment was set for an average one bond heteronuclear coupling of 150 Hz (3.3 ms). The long range delay utilised

to excite the heteronucleic multiple-quantum coherence was set for 8.3 Hz (60 ms). The spectrum was acquired as 2K x 267 data points, zero filled and subjected to both Fourier transforms to afford the 1024 x 1024 point data matrix. The number of transient per t_1 increment was 32. Spectra widths were 25062 Hz in F_1 (^{13}C) and 5841 Hz in F_2 (^{1}H). The 90° pulse widths were 9.33 µs (^{1}H) and 10.4 µs (^{13}C) and a 1 s interpulse delay was employed.

7-Methylisatin (Chen and Deady, 1993), benzothiophen-3(2*H*)one (Stridsberg and Allenmark, 1974) and 3-acetoxy-1-acetylindole (Railenau *et al*, 1967 & 1968) were prepared as reported in the literature. Ethyl salicylate and ethyl bromoacetate were reacted as reported in the literature (Schroeder *et al*, 1962), and the reaction worked up by an alternative procedure (Merriman, 1911) to give ethyl O-carbethoxymethyl-salicylate. This was then converted to benzofuran-3(2*H*)one (**27**) as previously reported (Schroeder *et al*, 1962).

1-Indanone, 6-methoxy-1-indanone, 6-methyl-1-indanone, 5,6-dimethoxy-1-indanone and 5-chloro-1-indanone were Aldrich chemicals. 5-Methoxy-1-indanone (from 5-indanol) (Panetta and Bunce, 1961) and 1-acenaphthenone (from 1-naphthylacetic acid) (Bosch and Brown, 1968) were prepared as reported. A minor modification was made to the reported preparation of 7-methyl-1-indanone (Thorsett and Stermitz, 1972). On the scale reported, the use of 200 µL of conc. HCl and 4 mL EtOH (total, added in one portion at the start) gave an improved yield in our hands. This method was also applicable to the preparation of 7-chloroindanone (**29i**), with an important modification. The initial Mannich reaction with 2-chloroacetophenone was as reported (Markovac-Prpic *et al*, 1960), but then, quaternization with methyl iodide and elimination to the substituted 2-propen-1-one occurred in the one pot under mild conditions. This intermediate was cyclized as for the 7-methyl analogue. 7-Methoxy-1-indanone was prepared from chroman-4-one by minor modification of a literature method

(Loudon and Razdan, 1954). The chromanone was added to the melt of aluminium trichloride and sodium chloride at 160°C, the temperature was raised to 200°C over 20 min and maintained at this temperature for a further 10 min. The mixture was poured into ice/conc. hydrochloric acid and filtered to give crude 7-hydroxy-1-indanone as a black solid. Without purification, this was methylated as reported (Loudon and Razdan, 1954) to give 7-methoxy-1-indanone, mp 102-103°C, in 45% yield. 4-Methoxyindan-1-one, mp 102-103°C (from ethanol) was prepared from dihydrocoumarin in 36% yield by the same two-step procedure as for the 7-methoxy isomer.

Preparation of 7-Chloro-1-indanone (**29i**)

The hydrochloride salt of *3-(dimethylamino)-1-(2-chlorophenyl)propan-1-one* was prepared as reported (Markovac-Prpic *et al*, 1960), and the free base was isolated as a golden oil in 59% yield. ^{1}H NMR ($CDCl_3$) d 2.18 (s, 6 H, $N(CH_3)_2$), 2.65 (t, 2 H, CH_2), 3.06 (t, 2 H, CH_2), 7.25-7.45 (m, 4 H, ArH).

A solution of this oil (20 g) and methyl iodide (30 mL) in benzene (200 mL) was allowed to stand at 4°C for 16 h. The solid which formed was filtered off and the solvent was removed from the filtrate under reduced pressure to give *1-(2-chlorophenyl)-2-propen-1-one* as a golden oil (8.6 g, 62%), suitable for further reaction. ^{1}H NMR ($CDCl_3$) d 6.07 (dd, 2 H, CH_2), 6.72 (dd, 1 H, CH), 7.25-7.4 (m, 4 H, ArH).

This compound was cyclized as for 7-methyl-1-indanone (Thorsett and Stermitz, 1972), except that the addition took 3 h. The product was recrystallized from light petroleum (bp 60-90°C) to give 7-chloro-1-indanone (14%), mp 95-96°C [Lit. 98°C (Kenner and Whitham, 1921)].

Amine linkers for bisamides

Diethylenetriamine, *N,N'*-bis(2-aminoethyl)-1,3-propanediamine, 1,4-bis(3-aminopropyl)piperazine,

spermidine, N,N'-bis(3-aminopropyl)-1,3-propanediamine and 3,3'-diamino-*N*-methyldipropylamine were used without further purification. The free base of triethylenetetramine was prepared from its hydrochloride (Glerup *et al*, 1970). O,O'-1,6-Hexanediylbishydroxylamine dihydrochloride was prepared as reported (Fuller and King, 1947).

Preparation of N,N'-Bis(2-aminoethyl)-N,N'-dimethyl-1,2-ethanediamine

A mixture of chloroacetonitrile (2.5 g, 28.4 mmol), *N,N'*-dimethylethylenediamine (4.4 g, 58.2 mmol) and anhydrous K_2CO_3 (14 g) in dry acetone (250 mL) was stirred and refluxed for 24 h. The solid was filtered off, washed with CH_2Cl_2 and the solvent removed from the filtrate under reduced pressure to give the intermediate bisacetonitrile (3.95 g, 84%) as a golden oil, which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 6 H, NCH_3), 2.61 (s, 4 H, NCH_2), 3.60 (s, 4 H, CH_2CN).

Diborane was prepared *in situ* (Brown and Subba Rao, 1960) and, with a nitrogen carrier gas, was bubbled through a solution of the bisacetonitrile (2.6 g) in tetrahydrofuran at room temperature over *ca.* 1 h (exothermic). This mixture was allowed to stir for a further 1 h, then EtOH was added cautiously to destroy the excess diborane before hydrogen chloride gas was passed into the solution. The salt which formed was filtered off, dissolved in water, the pH was taken to 12 with 10% NaOH, and the solvent was removed under reduced pressure. The residue was extracted with hot toluene, and the solvent was removed under reduced pressure to give the title compound (1.4 g, 53%), which was sufficiently pure to be used in amide formation. ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 6 H, NCH_3), 2.36 (t, 4 H, J = 6 Hz, CH_2), 2.42 (s, 4 H, CH_2), 2.68 (t, 4 H, J = 6 Hz, CH_2). ESMS: *m/z* 175.1 (100%) [(M + 1)/1].

N,N'-Bis(2-aminoethyl)-N,N'-dimethyl-1,3-propanediamine

The same two step sequence with N,N'-dimethyl-1,3-propanediamine gave the intermediate bisacetonitrile (91%) [^{1}H NMR ($CDCl_3$) δ 1.59 (m, 2 H, C-CH_2), 2.33 (s, 6 H, NCH_3), 2.48 (t, 4 H, J = 7 Hz, NCH_2), 3.50 (s, 4 H, CH_2CN)] and final tetraamine (55%). ^{1}H NMR ($CDCl_3$) δ 1.54 (m, 2 H, C-CH_2), 2.12 (s, 6 H, NCH_3), 2.25-2.35 (m, 8 H, CH_2), 2.67 (t, 4 H, J = 6 Hz, CH_2). ESMS: *m/z* 189.3 (100%) [(M + 1)/1].

Example 1 Preparation of Quinoline Carboxylic Acid Intermediates according to Reaction Scheme 1

*Preparation of Isatin-7-carboxylic acid (**24a**)*

A solution of methyl anthranilate (31.5 g), chloral hydrate (35 g) and hydroxylamine hydrochloride (28.6 g) in conc. H_2SO_4 (25 g) and water (1.4 L) was heated at 95°C for 10 min, then kept at 4°C for 16 h. The cream isonitroso intermediate (29.8 g, 64%) was filtered off, washed with water and dried. This compound (15.0 g) was added, with stirring, in portions over 30 min to conc. H_2SO_4 (75 g) maintained at 60-65°C. The mixture was then heated at 95°C for 1 h and poured onto ice (600 g). The resulting brown solid was filtered, dissolved in 1 M NaOH solution, filtered, and the filtrate taken to pH 2 with conc. HCl to give **24a**, (8.4 g, 65%), mp 274-275°C (H_2O) (lit[12] mp 276-277°C).

Preparation of 5-Methoxyisatin-7-carboxylic acid (**24b**)

The literature preparation (Cragoe *et al*, 1953), which used conc. H_2SO_4 in the cyclization step, did not work well in our hands but close adherence to the following conditions gave consistent results. 5-Methoxy-2-nitrobenzoic acid (5 g) was dissolved in EtOH (75 mL) and hydrogenated in the presence of 10% palladium on carbon (0.5 g). This gave *5-methoxyanthranilic acid* (4.14 g, 84%), mp 147-149°C [Lit.147-148°C (Cragoe *et al*, 1953)].

5-Methoxy-2-[[(hydroxyimino)acetyl]aminobenzoic acid was prepared from this acid (Cragoe *et al*, 1953) and the compound (5.0 g) was added with stirring, in portions to 85% H_2SO_4 (30 g) maintained at 50-55°C. The mixture was then heated at 100°C for 2 h. and poured onto ice (200 mL). The resulting solid was filtered off, dissolved in 10% NaOH solution, filtered, and the filtrate acidified to pH 2 with conc. HCl to give the isatin (3.3 g, 74%), mp 236-240°C (dec with preliminary darkening) [Lit. 210°C (Cragoe *et al*, 1953)].

Preparation of 5-Chloroisatin-7-carboxylic acid (**24c**)

5-Chloro-2-nitrobenzoic acid was reduced by a literature procedure for the 4-chloro isomer (Hunn, 1923) to give *5-chloroanthranilic acid* in 85% yield, mp 205-206°C [Lit. 204°C (Bergman and Berkovic, 1961)]. A solution of this compound in boron trifluoride/MeOH complex (20 mL/g) was stirred and heated at reflux for 50 h, to give *methyl 5-chloroanthranilate* in 70% yield, mp 62-64°C [Lit. 68-69°C (Cragoe *et al*, 1953)]. The isonitroso intermediate was prepared as reported (Cragoe *et al*, 1953) from this ester in 67% yield, mp 210-212°C (Lit.[1] 219-221°C). This compound was cyclized as for **24a** but at 100°C for 2 h, to give **24c** (74%), mp 218-220°C (dec after preliminary darkening). ^{1}H NMR d 7.78 (d, J = 2 Hz), 8.13 (d, J = 2 Hz), 10.44 (s, 1 H, NH), 13.91 (s, 1 H, CO_2H).

*Preparation of 11H-Indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33a**): Example of the General Pfitzinger Reaction (Method A)*

Isatin-7-carboxylic acid (**24a**) (1.82 g, 9.53 mmol) was added with stirring to 10% NaOH solution (30 mL), at 90°C under a nitrogen atmosphere. To this was added 1-indanone (**29a**) (0.8 g, 6.03 mmol) in small portions and the solution was heated and stirred for a further 1 h, cooled, then filtered. The filtrate was taken to pH 5 with conc. HCl, and a mixture of free acid and its sodium salt

separated (unreacted **24a** remained in solution). This was filtered off, stirred in hot water (most dissolved), and the pH taken to 2 with conc. HCl to give the product as a pale yellow solid (1.0 g, 54%), mp 295-298°C (with decarboxylation). ^{1}H NMR δ 4.31 (s, 2 H, CH_2), 7.58-7.69 (m, 2 H), 7.76-7.88 (m, 2 H), 8.10 (d), 8.56 (d), 8.70 (d).

The following acids were prepared in the manner described:

*4-Methylbenzothieno[3,2-b]quinoline-11-carboxylic acid (**35**)* was prepared by reaction of 7-methylisatin **25** and benzo[*b*]-thiophene-3(2*H*)-one (**28**) at 100°C, under N_2, for 6 h. A blue/mauve sodium salt separated on cooling (the filtrate gave some **28** at pH 6 and some **25** at pH 2) and this, dissolved in hot water, gave the free acid **35** at pH 5 (concentrated HCl), in 41% yield: mp 294-296°C (with decarboxylation). ^{1}H NMR δ 2.87 (s, 3 H, CH_3), 7.55-7.68 (m, 4 H), 8.03 (d), 8.47 (d), 8.76 (d).

*Benzothieno[3,2-b]quinoline-4,11-dicarboxylic acid (**32**)* was prepared by reaction of **24a** and **28** at 100°C, under N_2, for 8 h. A sodium salt separated at pH 6 (concentrated HCl) and this, dissolved in hot water, gave free acid **32** at pH 2, in 55% yield: mp 282-283°C (with decarboxylation). ^{1}H NMR δ 7.60 (t), 7.72 (t), 7.84 (t), 8.03 (d), 8.25 (d), 8.48 (d), 9.12 (d).

*Benzofuro[3,2-b]quinoline-4,11-dicarboxylic acid (**31**)* was prepared by reaction of **24a** and benzofuran-3(2*H*)one (**27**) under reflux for 6 h. The mixture was then cooled and filtered to remove a base insoluble by-product. The sodium salt of the product was largely soluble, and acidification of the filtrate to pH 2 with concentrated HCl gave a 5:2 mixture of the free acid (**31**) along with some unreacted **24a** which could not be separated (0.19 g from 0.3 g of **27**). This was treated further by dissolution in 6% Na_2CO_3 solution and reacidified to give a solid mixture which was decarboxylated as detailed below. ^{1}H NMR δ 7.60 (t), 7.80-7.93 (m, 3 H), 8.36 (d), 8.47 (d), 8.63 (d).

1-Methoxy-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33b**) was prepared from (**24a**) and 4-methoxy-1-indanone (**29b**), under nitrogen, for 1 h. Workup as for (**33a**) gave a bright yellow solid (72%), mp 314-316°C. ^{1}H NMR δ 3.88 (s, 3 H, OCH_3), 3.91 (s, 2 H, CH_2), 7.16 (d), 7.50 (d), 7.55 (t), 7.69 (t), 8.48 (d), 8.58 (d).

2-Methoxy-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33c**) was prepared from (**24a**) and 5-methoxy-1-indanone (**29c**), under nitrogen, for 1 h. Workup as for (**33a**) gave a yellow solid (74%), mp 312-315°C. ^{1}H NMR δ 3.84 (s, 3 H, OCH_3), 4.10 (s, 2 H, CH_2), 7.04 (d), 7.17 (s), 7.72 (t), 7.81 (d), 8.47 (d), 8.60 (d), 16.44 (s, COOH).

3-Methoxy-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33d**) was prepared from (**24a**) and 6-methoxy-1-indanone (**29d**), under nitrogen, for 1 h. Workup as for (**33a**) gave a yellow solid (66%), mp 315-317°C. ^{1}H NMR δ 3.88 (s, 3 H, OCH_3), 4.10 (s, 2 H, CH_2), 7.16 (d), 7.38 (s) 7.56 (d), 7.78 (t), 8.49 (d), 8.61 (d), 16.12 (s, COOH).

4-Methoxy-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33e**) was prepared from (**24a**) and 7-methoxy-1-indanone (**29e**), under nitrogen, for 2 h. Workup as for (**33a**) gave a yellow solid (64%), mp 285-287°C (dec. after preliminary darkening). ^{1}H NMR δ 4.04 (s, 3 H, OCH_3), 4.30 (s, 2 H, CH_2), 7.15 (d), 7.30 (d), 7.58 (t), 7.82 (t), 8.58 (d), 8.71 (d).

4-Methyl-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33g**) was prepared from (**24a**) and 7-methyl-1-indanone (**29g**), under nitrogen, for 8 h. Workup as for (**33a**) gave a light yellow solid (64%), mp 300-302°C (with decarboxylation). ^{1}H NMR δ 2.84 (s, 3 H, CH_3), 4.28 (s, 2 H, CH_2), 7.36 (d), 7.50-7.62 (m, 2 H), 7.84 (t), 8.57 (d), 8.67 (d).

Acenaphtho[1,2-b]quinoline-8,12-dicarboxylic acid (**33k**) was prepared from (**24a**) and 1-acenaphthenone (**29k**),

under nitrogen, for 1 h. Workup as for (**33a**) gave a yellow solid (64%), mp 330-338°C. ^{1}H NMR δ 7.74 (t), 7.79 (t) 7.91 (t), 8.08 (d), 8.25 (d), 8.29 (d), 8.38 (d), 8.42 (d), 8.53 (d).

4-Chloro-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33i**) was prepared by reaction of (**24a**) and 7-chloro-1-indanone (**29i**). Workup as for (**33a**) gave the product as a fawn solid (78%), mp 295-298°C (dec). ^{1}H NMR d 4.16 (s, 2 H, CH_2), 7.40 (d), 7.47 (t), 7.57 (d), 7.76 (t), 8.51 (d), 8.61 (d).

8-Methoxy-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33m**) was prepared by reaction of (**24b**) and 1-indanone (**29a**). Workup was as for (**33a**) and the crude solid was stirred with ethanol and filtered to give the dark red/tan product (66%), mp > 300°C, which contained *ca* 15% of the starting isatin. ^{1}H NMR d 3.82 (s, 3 H, OCH_3), 3.96 (s, 2 H, CH_2), 7.41-7.57 (m, 3 H), 7.72 (d), 7.85-7.88 (m, 2 H).

6H-Indeno[2,1-b]quinoline-4,11-dicarboxylic acid (**45**) was prepared by reaction of (**24a**) and 2-indanone (**44**) and the crude product was used in the next oxidation step without further treatment.

*Preparation of 3-Methyl-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33f**): Example of the General Pfitzinger Reaction (Method B).*

Isatin-7-carboxylic acid (**24a**) (2.5 g, 13.08 mmol) was added with stirring to 10% NaOH solution (40 mL), at reflux under a nitrogen atmosphere. To this was added portionwise a solution of 6-methyl-1-indanone (**29f**) (1.0 g, 6.84 mmol) in EtOH (40 mL) and the solution was heated and stirred for a further 3 h. The solution was cooled, concentrated to half the volume under reduced pressure and filtered. The filtrate was treated as in Method A to give the product as a pale yellow solid (1.25 g, 58%), mp 288-290°C (with decarboxylation). ^{1}H NMR δ 2.43 (s, 3 H,

CH_3), 4.12 (s, 2 H, CH_2), 7.38 (d), 7.53 (d), 7.74-7.83 (m, 2H), 8.52 (d), 8.63 (d).

The following acids were prepared in this manner:

2,3-Dimethoxy-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33j**) was prepared from (**24a**) and 5,6-dimethoxy-1-indanone (**29j**). Workup as for (**33f**) gave an orange solid (41%), mp >280°C (dec). ^{1}H NMR δ 3.84-3.87 (m, 6 H, OCH_3), 3.96 (s, 2 H, CH_2), 7.14 (s), 7.21 (s), 7.67 (t), 8.44 (d), 8.53 (d).

2-Chloro-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33h**) was prepared from (**24a**) and 5-chloro-1-indanone (**29h**). Workup as for (**33f**) gave a yellow solid (62%), mp >295°C (dec). ^{1}H NMR δ 4.23 (s, 2 H, CH_2), 7.64 (d), 7.78 (t), 7.87 (s), 8.09 (d), 8.54 (d), 8.64 (d).

8-Chloro-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**33n**) was prepared by reaction of 5-chloroisatin-7-carboxylic acid (**24c**) (1 mol) and 1-indanone (**29a**) (1.16 mol) for 8 h. This solid was extracted with hot EtOH and the insoluble brown solid (1.8 g from 2.5 g of **24c**) was a 4:1 mixture of **33n** and **24c**, suitable for the oxidation detailed below. ^{1}H NMR d 4.16 (s, 2 H, CH_2), 7.58-7.7 (m, 2 H), 7.76 (d), 8.08 (d), 8.37 (d, J = 2 Hz, 1 H), 8.77 (d, J = 2 Hz, 1 H).

*10H-Quindoline-4,11-dicarboxylic acid (**30**)*

A cooled solution of **24a** (1.6 g, 8.4 mmol) in KOH (28.8 g) and water (120 mL) was run into a flask containing 3-acetoxy-1-acetylindole (**26**) (0.8 g, 3.9 mmol) under an atmosphere of nitrogen, and the mixture shaken until solution was complete. The flask was sealed and stored in the dark for 10 days. Water (60 mL) was added, the green-yellow solution was heated, and oxygen was passed through for 20 min. The solution was filtered while hot to remove indigo, and the filtrate was acidified to pH 4 with conc. HCl to give 0.80 g of crude product. This was dissolved in

6% Na_2CO_3 solution, filtered, and the filtrate taken to pH 6 to give the free acid **30** as a red solid (0.24 g, 21.5%), mp softens at 280°C and decarboxylates 290-295°C. ^{1}H NMR δ 7.33 (t), 7.69-7.79 (m, 3 H), 8.23 (d), 8.44 (d), 9,23 (d), 11.63 (s, NH).

<u>Example 2</u> <u>Preparation of 11-Oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid</u> (**42a**)

Example of the General Decarboxylation Procedure.

The finely ground diacid (**34a**) (0.5 g) was placed in a cold finger sublimation apparatus at 0.5 mmHg and gently heated with a Bunsen burner until decarboxylation was complete (*c.* 5 min). The sublimate which formed was collected to give the product as light yellow needles (0.31 g, 72%), mp 357-359°C. ^{1}H NMR δ 7.72 (t), 7.77-7.91 (m, 3 H), 8.06 (d), 8.42 (d), 8.49 (d), 8.69 (s).

The following acids were prepared in this manner from the corresponding diacid:

3-Methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42f**). A yellow solid (69%), mp >295°C. ^{1}H NMR δ 2.52 (s, 3 H, CH_3), 7.52 (d), 7.75 (d), 7.80 (t), 7.88 (s), 8.44 (d), 8.50 (d), 8.85 (s).

4-Methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42g**). A light yellow solid (56%), mp >300°C. ^{1}H NMR δ 2.81 (s, 3 H, CH_3), 7.55-7.75 (m, 3 H), 7.81 (t), 8.41 (d), 8.50 (d), 8.85 (s).

2-Chloro-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42h**). A yellow solid (79%), mp >300°C. ^{1}H NMR δ 7.82 (t), 7.90-7.95 (m, 2 H), 8.10 (d), 8.43 (d), 8.49 (d), 8.91 (s).

4-Hydroxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42l**). A yellow solid (56%), mp >295°C. ^{1}H NMR δ 7.24 (d), 7.33 (d), 7.53 (t), 7.80 (t), 8.41 (d), 8.59 (d), 8.83 (s), 11.41 (s, 1 H, OH), 16.50 (s, 1 H, CO_2H).

1-Methoxy-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**41b**). A light yellow solid (75%), mp 260-265°C (from ethanol). ^{1}H NMR δ 3.94 (s, 3 H, OCH_3), 4.04 (s, 2 H, CH_2), 7.24 (d), 7.57 (t), 7.67 (d), 7.78 (t), 8.35 (d), 8.53 (d), 8.78 (s), 16.59 (s, 1 H, CO_2H).

2-Methoxy-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**41c**). A cream solid (70%), mp 171-175°C (from ethanol). ^{1}H NMR δ 3.87 (s, 3 H, OCH_3), 4.08 (s, 2 H, CH_2), 7.16 (d), 7.32 (s), 7.73 (t), 7.97 (d), 8.29 (d), 8.50 (d), 8.65 (s), 16.6 (s, 1 H, CO_2H).

3-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42d**). A yellow solid (43%), mp >295°C. ^{1}H NMR d 4.0 (s, 3 H, OCH_3), 7.20 (d), 7.46 (s), 7.8-7.85 (m, 2 H), 8.40 (d), 8.47 (d), 8.80 (s).

4-Chloro-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42i**). A fawn solid (70%) (with a trace amount of a minor component), mp >300°C. ^{1}H NMR d 7.72 (t), 7.80-7.95 (m, 3 H), 8.49 (d), 8.65 (d), 8.99 (s).

2,3-Dimethoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42j**). A yellow solid (15%) mp >310°C (from DMSO). ^{1}H NMR d 3.93 (s, 3 H, OCH_3), 4.04 (s, 3 H, OCH_3), 7.36 (s), 7.46 (s), 7.49 (t), 8.34 (d), 8.46 (d), 8.66 (s).

8-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42m**). A yellow solid (65%), mp 235-245°C (dec). ^{1}H NMR – too insoluble.

3-Hydroxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42o**). The sublimate was extracted with hot EtOH, and the yellow insoluble material was the product (42%), mp >295°C. ^{1}H NMR d 6.98 (d), 7.30 (s), 7.63 (d), 7.73 (t), 8.25-8.31 (m, 2 H), 8.66 (s).

6-Oxo-6H-indeno[2,1-b]quinoline-4-carboxylic acid (**47**). The sublimate was recrystallized from DMSO to give the product (51%), mp >300°C (from DMSO). ^{1}H NMR (75°C) d 7.55 (t), 7.77-7.90 (m, 3 H), 8.03 (d), 8.29 (d), 8.40 (d), 8.87 (s).

Acenaphtho[1,2-b]quinoline-8-carboxylic acid (**41k**). A yellow solid (57%), mp 158-160°C. ^{1}H NMR δ 7.64 (t), 7.76 (d) 7.84 (t), 7.88 (d), 8.08-8.11 (m, 2 H), 8.17 (d), 8.28 (d) 8.35 (d), 8.93 (s).

Example 3 Preparation of Further Quinoline Carboxylic Acids

*Benzothieno[3,2-b]quinoline-4-carboxylic acid (**40**)*

Finely ground diacid **32** (0.11 g) was heated on a hot-stage until it decarboxylated and liquefied (*c* 280°C). After 5 min. the vessel was cooled and the residue was extracted into 1M NaOH solution, filtered, and the filtrate acidified (pH 2, concentrated HCl) to give (**40**) as a brown solid (0.09 g, 95%), mp 281-283°C. ^{1}H NMR δ 7.61 (t), 7.70-7.80 (m, 2 H), 8.08 (d), 8.27-8.32 (m, 2 H), 8.53 (d), 9.24 (s).

8-Chloro-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42n**).

A black solid (89%) mp >300°C, which was used in the amidation reaction without further treatment. ^{1}H NMR—poorly resolved.

*4-Methylbenzothieno[3,2-b]quinoline (**37**)*

Finely ground acid 35 was heated at 300°C at 20 mmHg pressure for 5 min. The residue was extracted three times with hot acetone and the acetone was removed to give **37** as a reddish brown solid (96% yield), mp 124-126°C. The product can be further purified to a yellow crystalline solid by vacuum sublimation. ^{1}H NMR δ 2.86 (s, 3 H, CH_3), 7.53 (t), 7.59-7.68 (m, 3 H), 7.86 (d), 8.06 (d), 8.52 (d), 8.90 (s).

*11H-Indeno[1,2-b]quinoline-6-carboxylic acid (**41a**)*

Acid **33a** was heated at 295-300°C for 5 min. and the product **41a** (60% yield) was recrystallized from CH_2Cl_2/light petroleum (bp 90-110°C). ^{1}H NMR δ 4.16 (s,

2 H, CH_2), 7.58-7.64 (m, 2 H), 7.73-7.78 (m, 2 H) 8.06 (d), 8.33 (d), 8.52 (d), 8.72 (s).

*Benzofuro[3,2-b]quinoline-4-carboxylic acid (**39**)*

The 5:2 mixture of **31** and **24a** obtained above (0.19 g) was heated at 245°C for *c*. 5 min. The residue was stirred with warm EtOH which dissolved **24a**, and the insoluble product **39** (0.07 g), mp 250-255°C, was filtered off. ^{1}H NMR δ 7.60 (t), 7.82-7.92 (m, 3 H), 8.41 (d), 8.47 (d), 8.54 (d), 8.96 (s).

*10H-Quindoline-4-carboxylic acid (**38**)*

Diacid **30** was heated to 310°C until gas evolution ceased and the product (**38**) was left as yellow needles in 93% yield: mp > 310°C. ^{1}H NMR δ 7.33 (t), 7.62-7.71 (m, 3-H), 8.31 (d), 8.40 (d), 8.49 (d), 8.63 (s), 11.90 (s, NH).

*Preparation of 11-Oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34a**): Example of a General Oxidation Reaction*

Compound (**33a**) (3.0 g) was added to a solution of Na_2CO_3 (3.0 g) in water (120 mL) with stirring, at 55°C until the acid was dissolved. Potassium permanganate (3.6 g) was then added and the mixture was heated and stirred for *ca*. 10 min. (until a spot of reaction mixture on filter paper gave no pink color), then filtered through Celite, washed with 10% Na_2CO_3, then water and the filtrate was acidified to pH 2 with conc. HCl. The solid which formed was filtered to give the product as a yellow solid (2.5 g, 80%), mp >300°C. ^{1}H NMR δ 7.74 (t), 7.83-7.91 (m, 3 H), 8.07-8.15 (m, 2 H), 8.46 (d).

The following oxo acids were prepared in this manner:

*4-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34e**)*. A yellow solid (58%), mp >300°C,

from (**33e**). ^{1}H NMR δ 4.04 (s, 3 H, OCH_3), 7.41 (d), 7.50 (d), 7.67 (t), 7.86 (t), 8.15 (d), 8.63 (d).

3-Methyl-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (34f). A yellow solid (75%), mp 288-290°C (with decarboxylation), from (**33f**). ^{1}H NMR δ 2.52 (s, 3 H, CH_3), 7.53 (d), 7.75 (d), 7.84 (t), 7.89 (s), 8.10 (d), 8.45 (d).

4-Methyl-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (34g). A pale yellow solid (76%), mp 292-295°C (with decarboxylation), from (**33g**). ^{1}H NMR δ 2.84 (s, 3 H, CH_3), 7.60-7.75 (m, 3 H), 7.85 (t), 8.11 (d), 8.48 (d).

2-Chloro-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (34h). A yellow solid (with trace amount of starting material) (70%), mp 275-276°C, from (**33h**) (a 1.5 weight excess of Na_2CO_3 was used). ^{1}H NMR δ 7.82-7.95 (m, 3 H), 8.06 (d), 8.11 (d), 8.42 (d).

1-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (42b). A yellow solid (64%), mp 286-291°C, from (41b). ^{1}H NMR (80°C) δ 3.98 (s, 3 H, OCH_3), 7.29 (d), 7.53 (d), 7.75-7.8 (m, 2 H), 8.34 (d), 8.47 (d), 8.68 (s).

3-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34d**). A yellow solid (81%), mp >295°C. ^{1}H NMR d 3.96 (s, 3 H, OCH_3), 7.15 (d), 7.34 (s), 7.65-7.75 (m, 2 H), 7.98 (d), 8.39 (d).

4-Chloro-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34i**). An orange solid (67%), mp 290-292°C (with decarboxylation), from (**33i**). ^{1}H NMR d 7.72 (t), 7.80-7.95 (m, 3 H), 8.16 (d), 8.65 (d).

2,3-Dimethoxy-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34j**). An orange solid (77%) mp > 300°C, from (**33j**). ^{1}H NMR d 3.88 (s, 3 H, OCH_3), 3.99 (s, 3 H, OCH_3), 7.09 (s), 7.20 (s), 7.60 (t), 7.86 (d), 8.32 (d).

8-Chloro-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34n**). An orange solid (40%), containing

ca 15% (**33n**) by NMR) mp *ca* 270°C, from.(**33n**) ^{1}H NMR d 7.71 (t), 7.82-7.87 (m, 2 H), 7.95 (s), 7.98 (d), 8.25 (s).

6-Oxo-6H-indeno[2,1-b]quinoline-4,11-dicarboxylic acid (**46**). A tan solid, mp > 300°C, from (**45**). Yield, 63% for two steps from 2-indanone. ^{1}H NMR d 7.60 (t), 7.77-7.82 (m, 2 H), 7.86 (d), 7.91 (t), 8.14 (d), 8.32 (d).

*Preparation of 2-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylic acid (**42c**)*

Compound (**41c**) (0.5 g, 1.7 mmol) and glacial acetic acid (20 ml) were stirred and heated to reflux. Two drops of conc. sulphuric acid were added and the solution turned clear. The solution was removed from the heat, sodium dichromate (1.5 g, 5.0 mmol) was added carefully in small portions, and the mixture was refluxed for 1 h, then cooled to 4°C for 16 h. The solid which separated was filtered and a second crop was obtained from the filtrate, to give the product as a yellow solid (0.23g, 44%), mp 340-342°C (from ethylene glycol). ^{1}H NMR (80°C) δ 3.96 (s, 3 H, OCH_3), 7.37 (s) 7.39 (d), 7.75 (t), 7.98 (d), 8.33 (d), 8.47 (d), 8.72 (s).

*Preparation of Benzothieno[3,2-b]quinoline-4-carboxylic acid 10,10-dioxide (**43**)*

Concentrated H_2SO_4 (2.7 mL) was added dropwise with stirring to a cooled solution of compound 37 (0.97 g, 3.89 mmol) in HOAc (90 mL) and Ac_2O (27 mL). Chromium trioxide (9.0 g) was then added, and the mixture was stirred at room temperature for 1 h, then added to 100 mL of ice/water and taken to pH 4 with 50% NaOH solution. The resultant solid was filtered off and washed with cold acetone to give the product as a cream solid (0.42 g, 35%), mp >310°C.

Preparation of 8-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34m**)

A mixture of 33m (0.7 g), nickel peroxide (0.7 g)(Nakagawa *et al*, 1962) and sodium hydroxide (0.7 g) in water (20 mL) was stirred at room temperature overnight, then filtered through Celite and the filtrate was acidified to pH 2 (conc. HCl) to give a yellow precipitate. This was collected by filtration to give the product as a pale yellow solid (0.47 g, 65%) mp > 300°C. ^{1}H NMR d 3.93 (s, 3H, OCH_3) 7.28 (s), 7.67 (t) 7.79-7.85 (m, 2 H), 7.96 (d), 7.98 (s).

*Preparation of 4-Hydroxy-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34l**)*

Aluminium trichloride (2.0 g, 15 mmol) and sodium chloride (0.4 g, 6.8 mmol) were stirred and heated to 160°C under a nitrogen atmosphere. To this was added **34e** (0.4 g, 1.1 mmol) and the temperature was slowly raised to 195°C (*ca*. 10 min.) and then cooled to 180°C (*ca*. 10 min.). The resultant mixture was poured onto 10% HCl (60 mL), which was stirred and heated at 100°C for 2 h, then cooled. Filtration gave the product as a yellow solid (0.33 g, 86%), mp >300°C. ^{1}H NMR δ 7.28 (d), 7.34 (d), 7.56 (t), 7.86 (t), 8.15 (d), 8.66 (d), 11.54 (s, 1 H, OH), 16.35 (s, 1 H, CO_2H).

*Preparation of 3-Hydroxy-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxylic acid (**34o**).*

This was prepared in 65% yield from **34d**, as for **34l**, as a green solid, mp 295-298°C (with decarboxylation). ^{1}H NMR d 7.03 (d), 7.35 (s), 7.72 (d), 7.82 (t), 8.07 (d), 8.42 (d), 11.3 (br s, 1 H, OH).

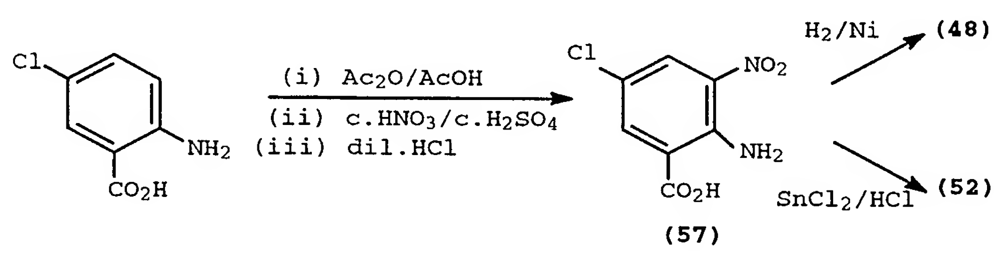
*Preparation of Methyl 4-Methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxylate (**36**)*

A mixture of hydroxyacid (**421**) (0.6 g, 2.1 mmol), silver (I) oxide (1.9 g, 8.2 mmol) and methyl iodide (9 mL)

in dry *N,N*-dimethylformamide (25 mL) was stirred at room temperature for 16 h. and then water (200 mL) was added. The solid which separated was filtered off, washed with water and dried. This was extracted (Soxhlet) with $CHCl_3$, and the $CHCl_3$ removed under reduced pressure to give the product (0.5 g, 76%), mp 165-167°C. ^1H NMR ($CDCl_3$) δ 4.09-4.11 (m, 6 H, OCH_3, CO_2CH_3), 7.20 (d), 7.46-7.52 (m, 2 H), 7.55 (d), 7.97 (d), 8.14 (d), 8.34 (s).

Example 4 Preparation of Quinoxaline-Based Acids **Precursors**

The NH dicarbonyl compound isatin (**55**) was used *per se*, and was also converted into *o*-hydroxyphenyl-glyoxylic acid (**56**) (Huntress and Hearon, 1941). This can be cyclized to benzofuran-2,3-dione (**53**) (Huntress and Hearon, 1941; Russell *et al*, 1970), but we successfully used (**56**) itself in the condensation reaction. The sulfur compound (**54**) was prepared from thiophenol and oxalyl chloride (Papa *et al*, 1949). A 4-step preparation of 2,3-diaminobenzoic acid (**48**) used an expensive starting material (Jones and Taylor, 1977) and a more recent synthesis was also lengthy (Denny *et al*, 1990). An alternative preparation, starting from the moderately priced 2-amino-5-chlorobenzoic acid, was devised, and is shown below. By suitable choice of reducing agent in the final step, both the dechloro (**48**) and chloro (**52**) diamines were accessible.



2-Amino-5-chloro-3-nitrobenzoic acid (57)

2-Amino-5-chlorobenzoic acid (Maybridge Chemical Co.) (5 g) was acetylated with a 1:1 mixture of acetic

anhydride (10 ml) and glacial acetic acid (10 ml) (refluxed for 0.5 h and poured into water) and the amide had m.p.190-192°. Nitration was carried out by adding the amide (2.0 g) in small portions over 45 min to a solution of concentrated nitric acid (4 ml) and concentrated sulphuric acid (4 ml) at 0°, and the mixture was stirred for a further 1 h then poured into 100 ml of ice-water. With continued stirring, the initially sticky precipitate gave a yellow solid. This was filtered off to give the nitroamide as a pale yellow powder (2.0 g), m.p. 195-197°. Hydrolysis of the amide was carried out by heating with 1:4 concentrated hydrochloric acid/water (50 ml) for 1 h, during which time a solid separated. The cooled solution was filtered to give (**57**) as yellow needles (1.40 g), m.p. 238-240°. ^{1}H n.m.r. [$(CD_3)_2SO/CDCl_3$] δ 8.10, s, 1H; 8.19, s, 1H.

*5-Chloro-2,3-diaminobenzoic acid (**52**)*

A mixture of compound (**57**) (2.3 g), stannous chloride dihydrate (9.0 g) and concentrated hydrochloric acid (30 ml) was stirred and heated at 100° until the clear solution was no longer yellow. The white precipitate which formed on cooling was collected by filtration to give a hydrochloride salt of the product (1.8 g), m.p. 195-200°, with decarboxylation. ^{1}H n.m.r. δ 7.23, d, J = 2.4 Hz, 1H; 7.47, d, J = 2.4 Hz, 1H.

*2,3-Diaminobenzoic acid (**48**)*

A mixture of compound (**52**) (0.5 g) and freshly prepared Raney nickel (Pavlic and Adkins, 1946) (2 g of ethanol wet material) in 0.2 M potassium hydroxide in ethanol (50 ml) was hydrogenated at atmospheric pressure. The catalyst was removed by filtration through celite, and the filtrate was concentrated to 5 ml, diluted to 30 ml with water and acidified to pH 2 with concentrated hydrochloric acid. The solvent was removed at reduced pressure to give the product as a hydrochloride salt in

mixture with potassium chloride (1.6 g). ^1H n.m.r. (D_2O) δ 6.74, t, H-5; 7.40, d, J = 7.9 Hz, H-4; 7.81, d, J = 8.2 Hz, H-6. This mixture was used in the condensation reactions given below.

Condensations

Previously reported reactions of benzofuran-2,3-dione (**53**) with 1,2-phenylenediamine have resulted in formation of compound (**58**) rather than the desired tetracycle (Logemann *et al*, 1963). We have now found that reaction in hot polyphosphoric acid (PPA) was quite successful, producing (**58**) only as a minor component. Only one isomer, (**49**), was formed from (**48**), while a 1:1 mixture of (**59B**)/(**59A**) was formed from (**52**) (see below for the assignments).

The reaction of isatin with 1,2-phenylene-diamine is complex, and Schiff base (**60**) and spiro compound (**61**), as well as the indolo[2,3-b]quinoxaline have been isolated, depending on conditions (Niume *et al*, 1982; Ivaschenko *et al*, 1984; Popp, 1969). In the present work, reaction in hot PPA brought about the desired reaction, with only a trace of spiro contaminant. Isomeric mixtures [4:1, (62B):(62A); 1:2, (64B):(64B)] were obtained in both cases, with the chloro substituent favouring (**Form A**), relative to hydrogen, as in the furo analogues.

Formation of the thieno system is simplest (Banerji *et al*, 1973), and there are no reports of other types of reaction products. This was also true in the present work, where both aqueous acetic acid and PPA were investigated as condensation media, with contrasting results. Reaction of (**54**) with (**48**) gave good yields of tetracyclic products; (**66A**) (5:1) was favoured in aqueous acetic acid, while the change to PPA reversed the preference and (**66B**) (3:1) predominated.

*[1]Benzofuro[2,3-*b*]quinoxaline-7-carboxylic acid (**49B**)*

A mixture of 2,3-diaminobenzoic acid/potassium chloride (1.6 g) (from 0.5 g (**57**)) and *o*-hydroxyphenylglyoxylic acid (Huntress and Hearon, 1941) (0.5 g) in polyphosphoric acid (15 g) was stirred and heated at 110° for 5 h, then cooled to room temperature and thoroughly mixed with water (100 ml). The green solid which separated was filtered off (0.39 g), washed with water and recrystallized from acetonitrile to give the product (0.2 g). For microanalysis, a sample was stirred with acetic acid/water (1:1), which removed the little (**58**), filtered and again recrystallized from acetonitrile, m.p. approx. 270° (after shrinking and darkening) (Found: C, 68.1; H, 2.9; N, 10.6. $C_{15}H_8N_2O_3$ requires C, 68.2; H, 3.1; N, 10.6).

*8-Chloro[1]benzofuro[2,3-*b*]quinoxaline-10-carboxylic acid (**59A**) and 9-chloro[1]benzofuro[2,3-*b*]quinoxaline-7-carboxylic acid (**59B**)*

5-Chloro-2,3-diaminobenzoic acid hydrochloride (0.44 g) and *o*-hydroxyphenylglyoxylic acid (Huntress and Hearon, 1941) (0.5 g) in polyphosphoric acid (15 g) were reacted as for (**49B**) to give a light green solid (0.24 g, 27%), m.p. 237-240° (from acetonitrile). This was shown by ^{1}H n.m.r. to be a 1:1 mixture of (**59A**) and (**59B**), containing a trace of the ring-open product which could not be removed. Electrospray mass spectrum: m/z 299 (100%), 300 (16), 301 (36), all (M+1).

*6*H*-Indolo[2,3-*b*]quinoxaline-4-carboxylic acid. (**62B**)*

2,3-Diaminobenzoic acid/potassium chloride (1.6 g) (from 0.5 g (**57**)) was combined with isatin (0.6 g) and polyphosphoric acid (15 g). The mixture was stirred and heated at 140° for 5 h, then cooled to room temperature and thoroughly mixed with water (200 ml). The solid which separated was filtered off and washed thoroughly with water, to give a black glass (0.75 g). This was treated

with 1M sodium hydroxide (50 ml), filtered, and the filtrate acidified to pH 2 with conc. hydrochloric acid. The product was collected by filtration to give 0.6 g of a dark solid. ^{1}H n.m.r. analysis suggested this was largely product, which could be further purified by Soxhlet extraction with tetrahydrofuran to give a yellow solid, m.p. > 300°. This contained the title compound and the isomeric (**62A**) in a 4:1 ratio, with trace impurities.

For microanalysis, a sample of the dark solid (0.35 g) in ethanol (12 ml) and concentrated sulphuric acid (2 ml) was heated under reflux for 2.5 h, then cooled, and the insoluble material was removed by filtration. The filtrate was concentrated at reduced pressure to 4 ml, diluted with water to 30 ml and the pH adjusted to 3 with 10% sodium hydroxide. The solid which separated was filtered off to give the ethyl esters (**63B**):(**63A**) (>19:1 by ^{1}H n.m.r.) (0.22 g), m.p. 228-230° (from ethanol) (Found: C, 70.2: H, 4.2: N, 14.2. $C_{17}H_{13}N_3O_2$ requires C, 70.1; H, 4.5; N, 14.4.)

*2-Chloro-6*H*-indolo[2,3-*b*]quinoxaline-4-carboxylic acid (**64B**) and 3-Chloro-6*H*-indolo[2,3-*b*]quinoxaline-1-carboxylic acid (**64A**)*

Reaction of 5-chloro-2,3-diaminobenzoic acid hydrochloride (**52**) (0.5 g) with isatin (0.6 g) and polyphosphoric acid (15 g) as for (**62B**) gave 0.6 g of a dark solid after the same base/acid treatment, which contained a 2:1 mixture of (**64A**):(**64B**). Extraction with hot tetrahydrofuran gave a yellow solid (0.22 g), m.p. > 300° with the same composition.

This was esterified as for (**62B**) to give a mixture of the ethyl esters as a tan solid, m.p. 220-225° (from ethanol/water) (Found: C, 62.4: H, 3.5: N, 12.7. $C_{17}H_{12}ClN_3O_2$ requires C, 62.7; H, 3.7; N, 12.9).

*[1]Benzothieno[2,3-*b*]quinoxaline-10-carboxylic acid (**66A**) and [1]benzothieno[2,3-*b*]quinoxaline-7-carboxylic acid. (**66B**)*

(a) A warm solution of 2,3-diaminobenzoic acid/KCl (1.6 g) in 1:1 acetic acid/water (10 ml) was added, with stirring, to a hot solution of benzothiophene-2,3-dione (Papa *et al*, 1949) (0.52 g) in glacial acetic acid (20 ml). The whole was warmed and stirred for 15 min. and the resultant precipitate was filtered off to yield a khaki solid (0.34 g, 38%)). ^{1}H n.m.r. analysis showed this to contain (**66A**) and (**66B**) (5:1), with m.p. range 240-261° after recrystallisation from ethanol (Found: C, 64.4; H, 3.1; N, 10.3. $C_{15}H_8N_2O_2S$ requires C, 64.3; H, 2.9; N, 10.0%).

(b) The conditions in PPA were as for (49B) to give a 75% yield of a mixture of (**66B**) and (**66A**) (3:1) as a green solid, m.p. 237-240°.

3-Phenylquinoxaline-5-carboxylic acid

A mixture of (**66A**)/(**66B**) (5:1, from acetic acid preparation above) (0.05 g), sodium carbonate (0.1 g), and deactivated Raney nickel (Spero *et al*, 1948) (5 g, acetone wet) in water (4 ml) was stirred vigorously and heated at 70° for 3 h. The catalyst was removed by filtration through celite, the filtrate was acidified to pH 2 with concentrated hydrochloric acid and the solid which formed was filtered off. This was recrystallized from ethanol to give a little unchanged tetracycle, and removal of the ethanol from the filtrate gave the product (0.015 g) as pale yellow needles, m.p. 206-208° (from methanol). ^{1}H n.m.r. ($(CD_3)_2SO$) δ 7.65-7.69, m, 4H; 7.97, t, 1H; 8.31-8.38, m, 4H; 9.74, s, H 2. ^{13}C n.m.r. ($(CD_3)_2SO$) δ 127.5, CH; 129.1, CH; 129.3, CH; 130.9, CH; 132.7, CH, 134.7, C; 138.3, C; 140.8, C; 144.4, CH; 150.1, C; 165.9, C. Electrospray mass spectrum: m/z 251 (M+1).

N-Oxidation procedure

A sample of the acid (approx. 0.08 g) was dissolved in hot glacial acetic acid (4 ml). Hydrogen peroxide solution (30%, v/v) (1.25 ml) was added and the whole was heated under reflux for the time indicated below, when substantial separation of solid had occurred. The mixture was cooled, and the solid filtered off and analysed by n.m.r. The following results were obtained.

From (**62A**)/(**62B**) (2 h). A mixture of (**62B**) N-oxide and unchanged (**62A**).

From (**64A**)/(**64B**) (2 h). A mixture of (**64A**) N-oxide and unchanged (**64A**).

From (**49B**) (2 h). Yellow needles of (**49B**) N-oxide, m.p. 269-270°.

From (**59A**)/(**59B**) (48 h). A mixture of (**59B**) N-oxide and unchanged (**59A**).

<u>Example 5</u> <u>Structural Assignment</u>

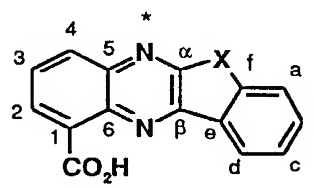
It was not possible to assign structures to the Form A or Form B series from ^{1}H or ^{13}C n.m.r. spectra, but this could be done for the O and NH compounds from the chemical shifts produced by N-oxidation.

The systematic numbering of the tetracycles depends on the isomer and the heteroatom, and comparisons are therefore confusing. For the n.m.r. discussion, therefore, the system shown in Table 2 has been adopted. In this, numbers refer to the A ring, starting at the acid substituted position, letters apply to the D ring, starting *ortho* to the heteroatom, and α and β refer to the 'inner' ring junction carbons, with α being that between the two heteroatoms. The difference between Form A and Form B compounds therefore lies in the relative positions of α and β with respect to the numbered positions. This cannot be ascertained from the n.m.r. spectra, although the atoms can be identified by n.m.r. as a necessary first step. The NH ethyl ester [later established as being (**63B**)] was a

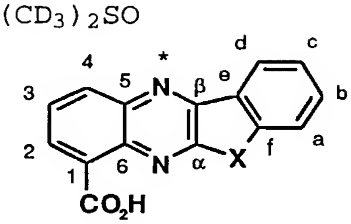
conveniently soluble reference compound for the ^{1}H and ^{13}C experiments required to achieve this.

Table 2

^{1}H n.m.r. data in $(CD_3)_2SO$



(**Form A**)



(**Form B**)

Compound	2	3	4	a	b	c	d
(**63B**)	8.00	7.74	8.38	7.55	7.72	7.37	8.35
(**49B**)	8.18	[A]	8.44	[A]	[A]	7.61	8.36
(**49B**) Nox	8.21	[A]	8.73	[A]	[A]	7.60	8.47
(**59A**)	8.15	-	8.35	[A]	[A]	7.61	8.34
(**59B**)	8.15	-	8.48	[A]	[A]	7.61	8.34
(**59B**) Nox	8.21	-	8.66	[A]	[A]	7.60	8.44
(**62A**)	8.37[B]	7.93	[C]	[C]	[C]	[C]	8.33[B]
(**62B**)	8.44	7.85	8.51	7.63	7.77	7.43	8.37
(**62B**) Nox	8.44	7.78	8.79	7.55	7.69	7.39	8.54
(**64B**)	8.23	-	8.52	7.61	7.77	7.42	8.35
(**64B**)	8.11	-	8.33	7.61	7.77	7.41	8.37
(**64B**) Nox	8.28	-	8.76	7.60	7.77	7.42	8.58
(**66A**)	8.32[B]	8.0	8.45	8.19	7.82	7.69	8.35[B]
(**66B**)	8.24	7.96	8.50	8.19	7.82	7.69	8.43

[A] 7.8-7.9, m

[B] May be reversed

[C] Peaks under those for major (**62B**)

The ^{1}H, ^{13}C, $^{1}J_{CH}$ HETCOR[14] and $^{3}J_{CH}$ HMBC[15] spectra for compound (**63B**) provided the data summarized in Table 3, which allow the atoms to be assigned without recourse to any reference spectra. For the carbons with attached protons, H-2 is the only one with $^{3}J_{CH}$ to the carbonyl carbon. There is also $^{3}J_{CH}$ to one other hydrogen bound carbon, which is therefore C-4. The remaining doublets for H-a and H-d can be distinguished from the extra $^{3}J_{CH}$ for the latter. The upfield triplet for H-c is assigned from the $^{3}J_{CH}$ to C-a. The close triplets for H-b and H-3 can be distinguished from the $^{3}J_{CH}$ of the former to H-d and the lack of $^{3}J_{CH}$ between H-3 and any other hydrogen bound carbon. The quaternary carbons may be assigned from $^{3}J_{CH}$ to protons already identified. This allows distinction between C-e ($^{3}J_{CH}$ to H-a and H-c) and C-f ($^{3}J_{CH}$ to H-b and H-d), C-5 ($^{3}J_{CH}$ only to H-3) and C-6 ($^{3}J_{CH}$ to H-2 and H-4), C-β ($^{3}J_{CH}$ only to H-d) and C-α (only one with no $^{3}J_{CH}$).

Table 3

CH coupling for compound (**63B**) in $(CD_3)_2SO$

^{13}C	CO	α	f	β	5	6	4	b	1	2	3	d	c	e	a	
^{1}H	167.3	145.8	144.4	140.4	138.3	137.6	132.0	131.8	131.0	128.5	125.0	122.6	121.0	118.9	112.2	
c	7.37 t													o	+	+
a	7.55 d													+	+	o
b	7.72 t			+					o				+			
3	7.74 t					+				+		o				
2	8.00 d	+					+	+			o					
d	8.35 d			+	+				+				o			
4	8.38 d						+	o			o					

o $^{1}J_{CH}$ (HETCOR) + $^{3}J_{CH}$ (HMBC)

Table 4

^{13}C n.m.r. data in $(CD_3)_2SO$[A]

Cpd	1 2	3	4	5	6	α	β	a	b	c	d	e	f	CO	
(63B)	131.0	128.5	125.0	132.0	138.3	137.6	145.8	140.4	112.2	131.8	121.0	122.6	118.9	144.4	167.3
(62B)	132.7	132.2	125.5	133.3	138.0	137.2	144.3	141.7	112.5	132.2	121.7	122.6	118.9	144.2	166.4
(62B) Nox	133.7	*133.2*	125.1	123.6	125.0	(139.8)		148.0	126.0	111.9	*131.3*	121.8	122.4	114.8	(140.6)
	166.5														
(49B)	131.6	130.3	127.9	132.0	140.4	136.4	155.1	141.1	113.1	133.5	125.1	123.1	120.6	158.3	165.8
(49B) Nox	131.5	*131.8*	127.0	120.4	135.6	138.1	158.1	124.8	111.6	*131.6*	124.6	123.0	116.5	154.3	165.8

[A] Pairs in italics, parenthesis, or underlined may be interchanged.

The remaining ^{1}H and ^{13}C assignments listed in Tables 2 and 4 were made by reference to those for (**63B**), aided by additional proton-coupled ^{13}C and $^{1}J_{CH}$ HETCOR experiments, and the effect of O relative to NH illustrated by spectra of dibenzofuran and carbazole (Black and Heffernan, 1965; Giraud and Marzin, 1979). Labelling as Form A or Form B required additional data from N-oxidation experiments.

Using quinoline (Barbieri *et al, 1975*; Su *et al*, 1978) and our previously reported compounds as examples (Deady *et al*, 1993; Deady and Quazi, 1995), N-oxidation results in downfield shifts in the ^{1}H spectrum for 'near' hydrogens, for Example, 4 and d for reaction at N^{*} in Form B. In ^{13}C spectra on the other hand, N-oxidation causes marked upfield shifts for quaternary carbons next to the N-O function, while the more distant ring junction carbons are hardly affected. In addition, quinoline N-oxidation is also accompanied by a substantial upfield shift for the peri C; C-4 is the equivalent in the tetracycles.

There are four possible structures for the N-oxide from a compound which could be Form A or Form B. However, in the present work, all N-oxides showed appropriate shifts for C-4 and H-4. Thus, the steric effect of the 1-CO_2H group restricted oxidation to N*, so that the possibilities were now reduced to the two structures shown, and the expectations from N-oxidation are:

Form A: upfield shifts for C-4, C-5, C-α; downfield shifts for H-4, H-d

Form B: upfield shifts for C-4, C-5, C-β; downfield shifts for H-4.

Though some signal overlap occurred in the ^{1}H n.m.r. spectra, the most downfield peaks, which included those for H-4 and H-d, were always distinguishable. As an example, inspection of the results from Tables 3 and 4 for the single compound formed in the dechloro O case indicates

N-oxidation shifts only compatible with this being (**49B**). This was true also for the NH analogue (**62B**).

Where mixtures of Form A and Form B isomers were present, the analysis was still reasonably straightforward, as only the Form B isomer reacted. So, for example, the mixture in the chloro NH case, after oxidation, showed distinguishable peaks for (**64B**) N-oxide and unchanged (**64A**).

The N-oxidation approach failed for the sulfur case. While there are examples of N-oxidation in compounds with adjacent N and S containing rings (Klemm *et al*, 1971), no success was achieved with the product of the acetic acid condensation described above [(**66A**) or (**66B**)]. Proton spectra were complex, but always showed upfield shifts, suggesting breakdown of the ring system. An alternative approach made use of the ready desulfurization of many compounds, including dibenzothiophen, with Raney nickel (Blicke and Sheets, 1949). The sulfur tetracycle, with deactivated catalyst and careful control of conditions (in order to avoid overreaction, afforded a phenylquinoxaline-carboxylic acid. This was characterized by the appearance of the hetero ring CH singlet in the ^{1}H n.m.r. spectrum, and the proton coupled ^{13}C spectrum allowed it to be identified as (**67**). In particular, C-4a (138.3 ppm) and C-8a (140.8) were assigned by reference to quinoxalines (McNab, 1982). The latter was a well resolved triplet ($^{3}J_{CH2,7}$ = 10.0 Hz), the former less so, and this multiplicity is incompatible with the alternative 2-phenyl isomer (C-8a should be a doublet). Thus the major tetracyclic product from the acetic acid condensation is (**66A**).

Example 6 Preparation of *N*-[2-(Dimethylamino)-ethyl]-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide (**12a**)

Example of the General Amidation Reaction: Method A

Freshly distilled Et_3N (0.13 g, 1.3 mmol) was added to a stirred suspension of the acid **42a** (0.29 g, 1.1 mmol) in CH_2Cl_2 (20 mL) in a nitrogen atmosphere. The resulting solution was taken to <-10°C and isobutyl chloroformate (0.18 g, 1.3 mmol) in CH_2Cl_2 (15 mL) was added dropwise over 0.75 h. After a further 0.5 h at this temperature, a solution of *N,N*-dimethylethylene-diamine (0.12 g, 1.3 mmol) in CH_2Cl_2 (15 mL) was added dropwise over 0.5 h. The solution was stirred at <-5°C for 0.5 h, 0°C for 1 h and room temperature for 1 h, then filtered and the filtrate was washed with a saturated solution of $NaHCO_3$ (3 x 30 mL), then with brine and water. The organic layer was dried ($MgSO_4$), the solvent was evaporated and the crude product was recrystallized from EtOH to give **12a** as white needles (0.25 g, 65%), mp 222-223°C. ^{1}H NMR δ 7.65-7.72 (m, 2 H), 7.79-7.84 (m, 2 H), 8.22-8.27 (m, 2 H), 8.62 (d), 8.68 (s), 10.71 (br s, NH). Anal. ($C_{21}H_{19}N_3O_2$) C, H, N.

The following *N*-[2-(dimethylamino)ethyl]-carboxamides were prepared in a similar manner:

N-[2-(Dimethylamino)ethyl]benzofuro[3,2-b]quinoline-4-carboxamide (**8**)

A pale yellow solid (24%), mp 105-107°C (from light petroleum (bp 90-110°C). ^{1}H NMR δ 7.49 (t) 7.61-7.71 (m, 3 H), 8.06 (dd, J = 7.9, 1.5 Hz), 8.23 (s), 8.45 (d), 8.88 (dd, J = 7.3, 1.5 Hz), 11.60 (s, NH). Anal. ($C_{20}H_{19}N_3O_2$) C, H, N.

*N-[2-(Dimethylamino)ethyl]benzothieno[3,2-b]quinoline-4-carboxamide (**9**)*

A red solid (51%), mp 137-139°C (from CH_2Cl_2/light petroleum (bp 90-110°C)), which could not be freed of trace

impurities. ^{1}H NMR δ 7.64-7.78 (m, 3 H), 8.12 (d), 8.23 (d) 8.70 (d), 8.94 (d), 9.19 (s), 11.18 (br s, NH).

*N,N'-Bis[2-(dimethylamino)ethyl]benzothieno[3,2-b]-quinoline-4,11-dicarboxamide **(10)***

A red solid (30%) mp 137-139°C (from MeCN). ^{1}H NMR δ 7.49-7.64 (m, 3 H), 7.83 (d), 8.30 (d) 8.72 (d), 8.82 (d), 11.22 (br s, NH). Anal. ($C_{25}H_{29}N_5O_2S$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-11H-indeno[1,2-b]quinoline-6-carboxamide **(11)***

Yellow needles (52%), mp 138-140°C (from toluene/light petroleum (bp 90-110°C)). ^{1}H NMR δ 4.14 (s, 2 H, CH_2) 7.57-7.60 (m, 2 H), 7.67 (t), 7.71 (d), 8.18 (d), 8.50 (d), 8.58 (s), 8.62 (d), 11.45 (br s, NH). Anal. ($C_{21}H_{21}N_3O$) C, H, N.

*N-[2-(Dimethylamino)ethyl]benzothieno[3,2-b]quinoline-4-carboxamide 10,10-dioxide **(13)***

Red needles (41%), mp 248-255°C (from EtOH). ^{1}H NMR δ 7.82-7.91 (m, 2 H), 8.03(t), 8.17(d), 8.33(d), 8.72 (d), 8.74(d), 9.37 (s), 10.45 (br s, NH). Anal. ($C_{20}H_{18}N_3O_3S$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-11-oxo-11H-indeno[1,2-b]-quinoxaline-6-carboxamide **(17)***

Yellow needles (61%), mp 220-221°C (from EtOH). ^{1}H NMR δ 7.77 (t), 7.90-8.05 (m, 3 H), 8.27 (d) 8.34 (d) 8.64 (d), 10.55 (br s, NH). Anal. ($C_{20}H_{18}N_4O_2$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-6-oxo-6H-indeno[2,1-b]quinoline-4-carboxamide **(19)***

Yellow needles (59%), mp 163-165°C (from toluene). ^{1}H NMR δ 7.46 (t), 7.62-7.74 (m, 3 H), 7.81 (d), 7.94 (d), 8.26 (s), 8.76 (d), 10.87 (br t, NH). Anal. ($C_{21}H_{19}N_3O_2$) C, H, N.

*N-[2-(Dimethylamino)ethyl]benzofuro[2,3-b]quinoxaline-7-carboxamide **(22)***

A pale tan solid (61%), after trituration with hexane, but which formed a sticky hydrate on standing. ^{1}H NMR ($CDCl_3$) δ 7.45 (t), 7.57-7.68 (m, 2 H), 7.79 (t), 8.21 (d), 8.29 (d), 8.79 (d), 10.14 (s, NH).

*N-[2-(Dimethylamino)ethyl]benzothieno[3,2-b]quinoxaline-10-carboxamide **(18)***

Pale yellow needles (66%), mp 208-210°C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 7.47 (t), 7.58 (t), 7.75 (d), 7.82 (t), 8.16 (d), 8.73 (d), 8.84 (d), 11.02 (s, NH). Anal. ($C_{19}H_{18}N_4OS$) C, H, N.

*N,N'-(Methyliminodi-3,1-propanediyl)bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] **(71)***

A pale orange semi-solid (85%). ESMS: m/z 660 (M+1), 330.7 ((M+2)/2). ^{1}H NMR ($CDCl_3$) δ 2.02 (m, 4H, $-CH_2-$), 2.39 (s, 3H, $N-CH_3$), 2.76 (t, 4H, J = 7.1 Hz, CH_2N), 3.62 (q, 4 H, J = 6.1 Hz, $CONH-CH_2$), 7.08-7.18 (m, 4H, H-1,2), 7.42 (t, 2H, J = 8 Hz, H-3), 7.51 (t, 2H, J = 7.8 Hz, H-8), 7.59 (d, 2H, J = 7.5 Hz, H-4), 7.81 (d, 2H, J = 7.8 Hz, H-9), 8.08 (s, 2H, H-10), 8.63 (d, 2H, J = 7.2 Hz, H-7), 10.58 (t, 2H, J = 4.9 Hz, NH). A hygroscopic perchlorate salt was prepared in 2-propanol and had mp 173-176°C.

*N, N'-(1,4-Piperazinediyldi-3,1-propanediyl)bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] **(72)***

From acid **42a** (1.1 mmol) and 1,4-bis(3-aminopropyl)-piperazine (0.5 mmol) as for **71**. The product separated from the reaction mixture and was obtained as a cream solid (47%), mp 280-282°C (from DMSO). ESMS: m/z 715 (M+1), 358 ((M+2)/2). ^{1}H NMR (DMSO-d_6) δ 1.85-1.95 (m, 2H, CH_2), 2.45-2.53 (m, 6H, pip CH_2, NCH_2), 3.59 (q, J = 5.6 Hz, $CONH-CH_2$), 7.65 (t, J = 7.7 Hz, H-2), 7.71 (t, J = 7.65 Hz, H-3), 7.78-7.84 (m, 2H, H-1,8), 8.06

(d, J = 7.3 Hz, H-4), 8.24 (d, J = 8 Hz, H-9), 8.52 (d, 1, J = 6.8 Hz, H-7), 8.68 (s, H-10). Anal. ($C_{44}H_{38}N_6O_4.H_2O$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-6H-indolo[2,3-b]quinoxaline-4-carboxamide (**20**)*

6*H*-Indolo[2,3-*b*]quinoxaline-4-carboxylic acid (**50**) was reacted with twice the mol ratio of other reagents described in the general method above to give the intermediate carbamate **51** as a tan solid (43%, >97% pure by NMR), mp >128°C (slow dec.) after trituration of the crude oil with hexane. ^{1}H NMR ($CDCl_3$) δ 1.12 (d, *J* = 6.7 Hz, 6 H, CH(CH_3)$_2$), 2.27 (m, 1 H, *C*H(CH_3)$_2$), 2.44 (s, 6 H, N(CH_3)$_2$), 2.89 (t, *J* = 6 Hz, 2 H, CH_2N), 3.85 (q, *J* = 6 Hz, 2 H, NHCH_2), 4.41 (d, *J* = 6.6 Hz, 2 H, OCH_2), 7.53 (t), 7.72 (t), 7.84 (t), 8.19 (d), 8.34 (d), 8.40 (d), 8.87 (d), 11.17 (br s, 1 H, NH).

A solution of aqueous NaOH (6 mL, 0.25 M) was added with stirring to a solution of **51** (0.1 g) in dioxan (20 mL), causing the solution to turn deep red. Stirring was continued for a further 16 h, when the solution was neutralized with HCl and the mixture was concentrated under reduced pressure to 5 mL. This was extracted with CH_2Cl_2 (3 x 10 mL), the combined extracts were dried ($MgSO_4$) and the solvent was removed to give **20** as a viscous yellow semi solid (0.06 g, 76%, >95% pure by NMR). ^{1}H NMR ($CDCl_3$) δ 7.05 (t), 7.21 (t), 7.29 (d), 7.54 (t), 7.91 (d), 7.94 (d), 8.02 (d), 11.13 (s, NH), 12.50 (s, NH). Attempts to purify this material for microanalysis were not satisfactory, and various salts were very hygroscopic.

*N-[2-(Dimethylamino)ethyl]-6H-3-chloroindolo[2,3-b]-quinoxaline-1-carboxamide (**14**) and N-[2-(dimethylamino)-ethyl]-6H-2-chloroindolo[2,3-b]quinoxaline-4-carboxamide (**21**)*

An isomeric mixture of precursor acids prepared according to Example 6 was reacted as for **20** to give the

intermediate carbamate mixture as a pale yellow solid (40%) after trituration of the crude oil with hexane. This mixture was hydrolysed as for **20**. In this case, all solvents were removed from the neutralized reaction mixture, water was added and the crude mixture of carboxamide isomers separated as an orange solid. This (0.1 g) was stirred with ice-cold $CHCl_3$ (1 mL) and filtered. Evaporation of the filtrate gave a sample of **14** (free of **21**) (0.03 g) mp 218-220°C. ^1H NMR ($CDCl_3$) δ 7.29 (d), 7.31 (t), 7.62 (t), 7.94 (s), 7.95 (s), 8.08 (d), 11.10 (s, N-H), 12.48 (s, NH). Alkaline hydrolysis gave the corresponding carboxylic acid. The solid from the first filtration was stirred with $CHCl_3$ (3 x 1 mL) and filtered each time, and the final insoluble solid was a sample of **20** (free of **14**) (0.028 g), mp 294-296°C. ^1H NMR [$CDCl_3$/$(CD_3)_2SO$] δ 7.32 (t), 7.50 (d), 7.62 (t), 8.12 (s), 8.38 (d), 8.49 (s), 11.15 (s, NH), 11.99 (s, NH). For microanalysis, a monoperchlorate salt of the amide mixture was prepared and had mp >270°C (slow dec.) after recrystallization from water. Anal. ($C_{19}H_{18}ClN_5O.HClO_4.0.5H_2O$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-11H-indeno[1,2-b]quinoxaline-6-carboxamide (**16**)*

11-Oxo-11*H*-indeno[1,2-*b*]quinoxaline-6-carboxylic acid (Deady *et al*, 1993) (0.2 g), ethylene glycol (40 ml), potassium hydroxide (0.88 g) and hydrazine hydrate (0.64 g) were heated at 140°C with stirring for 2 h. The condenser was removed and the temperature was increased gradually to 180°C over 1 h. The condenser was replaced and the solution was heated under reflux for 4 h. Water (40 mL) was added to the cooled solution which was then taken to pH 2 with conc. hydrochloric acid. The resulting precipitate was extracted into $CHCl_3$, the solution dried ($MgSO_4$), and the solvent removed *in vacuo* to give 11*H*-indeno[1,2-*b*]quinoxaline-6-carboxylic acid (0.12 g, 63%), sufficiently pure for amidation. A sample

recrystallized from EtOH had mp >250°C (slow dec.). ^{1}H NMR δ 4.24 (s, 2 H, CH_2), 7.56-7.70 (m, 2H), 7.78 (d) 7.90 (t) 8.14 (d), 8.29-8.33 (m, 2 H). This was reacted by the standard method to give the amide **16** as yellow needles (57%), mp 188-190°C [from light petroleum (bp 90-110°C)]. ^{1}H NMR ($CDCl_3$) δ 4.16 (s, 2 H, CH_2), 7.53-7.58 (m, 2 H), 7.67 (d), 7.78 (t), 8.20 (d), 8.43 (d), 8.85 (d), 11.14 (s, N-H). Anal. ($C_{20}H_{20}N_4O$) H, N; C: calcd, 72.3; found, 71.8.

Example 7 Preparation of *N,N'*-(Iminodi-2,1-ethanediyl)bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**69**).

Example of the General Amidation Reaction, Method B

Oxo acid **42a** (0.3 g) and 1,1'-carbonyldiimidazole (0.5 g) in dry dioxan (20 ml) were heated under reflux until dissolution was complete (*c* 3 h). The solvent was removed in vacuo and the residue was dissolved in dichloromethane (30 ml). The organic layer was washed twice with warm water (20 ml), and dried over $MgSO_4$. The solvent was removed to give 11-Oxo-11H-indeno[1,2-b]quinoline-6-carboxyimidazolide (**68**) as an orange/red solid (310 mg, 87%), mp 190-196°C (dec.). ^{1}H NMR ($CDCl_3$) δ7.11 (s, 1, H-4'), 7.48-7.53 (m, 2, H-2, 5'), 7.60 (t, 1, J = 6.7 Hz, H-3), 7.66 (t, 1, J = 7.7 Hz, H-8), 7.75 (d, 1, J = 7.5 Hz, H-1), 7.80 (d, 1, J = 7.3 Hz, H-4), 7.88 (s, 1, H-2'), 7.98 (d, 1, J = 7.0 Hz, H-9), 8.14 (d, 1, J = 8 Hz, H-7), 8.42 (s, 1, H-10).

To imidazolide **68** (0.4 g) in dry dichloromethane (30 ml) was added diethylenetriamine (0.063 g) and the whole was stirred at room temperature for 24 h., then washed with 10% sodium carbonate solution (2 x 20 ml), warm water (2 x 20 ml) and dried ($MgSO_4$). The solvent was removed to give the bisamide as a red solid (0.26 g, 69%), mp 245-247°C. ESMS: m/z 618 (M+1). ^{1}H NMR (DMSO-d_6) δ3.06 (s, CH_2-NHCO), 3.69 (s, CH_2-NH) 7.13-7.15 (m, 2H), 7.36-7.46 (m, 2H, H-8), 7.82 (d, J = 7.4 Hz, H-1), 7.95 (d,

J = 7.5 Hz, H-9), 8.16 (d, J = 7.3 Hz, H-7), 8.22 (s, H-10), 10.72 (br s, NH). Anal. ($C_{38}H_{27}N_5O_4.H_2O$) C, H, N.

The following carboxamides were made in a similar manner:

*N-[2-(Dimethylamino)ethyl]-4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12g**).*

The imidazolide from acid **42g** was not isolated but was reacted in CH_2Cl_2 solution with *N,N*-dimethylethylenediamine (1.3 mol/mol **42g**) to give the amide (70%), mp 186-187°C (from EtOH). ^{1}H NMR ($CDCl_3$) δ 2.92 (s, 3 H, CH_3), 7.40-7.55 (m, 2 H), 7.64 (t), 7.72 (d), 7.98 (d), 8.43 (s), 8.87 (d), 10.8 (s, 1 H, NH). Anal. ($C_{22}H_{21}N_3O_2.0.5H_2O$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-3-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12f**)*

This was prepared as for **12g** as a pale yellow solid (70%), mp 207-209°C (from EtOH). ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 3 H, CH_3), 7.34 (d), 7.61 (t), 7.72 (d), 7.93-7.97 (m, 2 H), 8.37 (s), 8.87 (d), 11.2 (s, 1 H, NH). Anal. ($C_{22}H_{21}N_3O_2$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-2-chloro-11-oxo-11H-indeno-[1,2-b]quinoline-6-carboxamide (**12h**)*

Twice the volume of 1,4-dioxane as in the general method was used, and the reflux time was 7 h. (with more 1,1'-carbonyldiimidazole (0.3 g) added after 4 h.). The intermediate imidazolide was then reacted as for **12g** to give the product as a pale yellow solid (90%), mp 236-238°C (from EtOH). ^{1}H NMR ($CDCl_3$) δ 7.60-7.65 (m, 2 H), 7.75 (s), 7.95 (d), 8.32 (d), 8.39 (s), 8.88 (d), 11.1 (s, 1 H, NH). Anal. ($C_{21}H_{18}ClN_3O_2$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-4-chloro-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12i**)*

A pale yellow solid (45%), mp 213-215°C (from MeCN). ^{1}H NMR ($CDCl_3$) d 7.46 (t), 7.60-7.70 (m, 2 H), 7.74 (d), 7.97 (d), 8.44 (s), 8.90 (d), 11.1 (s, 1 H, NH). Anal. ($C_{21}H_{18}N_3O_2Cl.0.5H_2O$: C, H, N.

*N-[2-(Dimethylamino)ethyl]-2,3-dimethoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12j**).*

A yellow solid (66%), mp 217-219°C (from MeCN). ^{1}H NMR d 7.57 (t), 7.68 (t), 7.84 (d), 7.90 (s), 8.30 (s), 8.35 (d), 8.81 (s), 11.09 (br s, NH). Anal. ($C_{23}H_{23}N_3O_4$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-8-methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12m**).*

Yellow needles (46%), mp 201-203°C (from MeCN). P14 ^{1}H NMR d 7.26 (s), 7.50 (t), 7.65 (t), 7.81 (d), 8.27-8.29 (m, 2 H), 8.54 (s), 11.24 (br s, NH). Anal. ($C_{22}H_{21}N_3O_3.0.5H_2O$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-8-chloro-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12n**).*

A tan solid (31%), mp 232-234°C (from MeCN). ^{1}H NMR d 7.57 (t), 7.68 (t), 7.84 (d), 7.90 (s), 8.30 (s), 8.35 (d), 8.81 (s), 11.09 (br s, NH). ESMS: m/z 380, 381, 382 (M+1). Anal. ($C_{21}H_{18}ClN_3O_2$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-3-hydroxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12o**).*

The imidazolide in dioxan solvent was prepared in the standard way. N,N-(Dimethylamino)ethylenediamine was added directly and the solution was stirred for 16 h. The solvent was removed under reduced pressure. The residue was dissolved in water and, after 1 h, the water was removed under reduced pressure. The residue was extracted with hot light petroleum (bp 60-90°C) and the insoluble material was

stirred with cold MeCN, filtered and recrystallized from ethanol to give the product as a yellow solid, mp 230-233°C, in 33% yield. ^{1}H NMR ($CDCl_3$) d 6.10 (d), 6.80 (s), 6.93 (d), 7.57 (t), 7.85 (d), 8.04 (s), 8.76 (d), 11.05 (br s, 1 H, NH).

*N-[2-(2-(Hydroxyethyl)amino)ethyl]-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**82**).*

This was prepared in 40% yield from imidazolide **68** and 2-(2-aminoethylamino)ethanol, as a red solid, mp 170-172°C (from MeCN). ^{1}H NMR d 2.93 (t, 2 H), 3.05 (t, 2 H), 3.70 (t, 2 H), 3.81 (q, 2 H), 7.56 (t), 7.63 (t), 7.72 (t), 7.84 (d), 7.98 (d), 8.11 (d), 8.42 (s), 8.87 (d), 11.17 (br s, NH). ESMS: m/z 362 (M+1). Anal.($C_{21}H_{19}N_3O_3$) H, N; C: calcd, 69.8; found: 69.2.

*N-[2-(Dimethylamino)ethyl]-1-methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12b**)*

An orange solid (61%), mp 194-197°C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 4.04 (s, 3 H, OCH_3), 7.04 (d), 7.60-7.69 (m, 2 H), 7.95-8.02 (m, 2 H), 8.42 (s), 8.84 (d), 11.3 (s, 1 H, NH).

*N-[2-(Dimethylamino)ethyl]-2-methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12c**)*

Bright yellow needles (47%), mp 199-201°C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 3.91 (s, OCH_3), 7.14 (dd), 7.25 (d, J = 2.3 Hz), 7.55 (t), 7.88 (d), 8.18 (d), 8.28 (s), 8.82 (d), 11.24 (s, 1 H, NH). Anal. ($C_{22}H_{21}N_3O_3$) C, H, N.

*N-[2-(Dimethylamino)ethyl]-3-methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12d**)*

A cream solid (52%), mp 219-221°C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 3.99 (s, OCH_3), 6.98 (dd), 7.62 (t), 7.77-7.81 (m, 2 H), 7.98 (d), 8.36 (s), 8.85 (d), 11.24 (s, 1 H, NH). Anal. ($C_{22}H_{21}N_3O_3$) C, H, N.

*N-[2-(Dimethylamino)ethyl]acenaphtho[1,2-b]quinoline-8-carboxamide (**12k**)*

A pale yellow solid, mp 122-124°C (from MeCN). ^{1}H NMR ($CDCl_3$) d 7.55-7.8 (m, 3 H), 7.90-8.05 (m, 4 H), 8.45-8.55 (m, 2 H), 8.84 (d), 11.7 (br s, 1H, NH). Anal. ($C_{24}H_{21}N_3O$) C, H, N.

*N,N'-[[(2-Aminoethyl)imino]di-2,1-ethanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**70**)*

This was prepared from imidazolide **68** and triethylenetetramine as for **69** but the reaction time was 72 h. The required bisamide was an orange solid (73%), mp 181-184°C (from DMSO). ESMS: m/z 661 (M+1). ^{1}H NMR (DMSO-d_6) δ 2.80-2.88 (m, 6H, CH_2-NH), 7.37-7.41 (m, 2H), 7.55-7.65 (m, 2H), 8.05-8.15 (m, 2H), 8.32 (s, H-10), 8.42 (d, J = 7.0 Hz, H-9), 10.61 (br s, NH). Anal. ($C_{40}H_{32}N_6O_4.2.5H_2O$) C, H, N.

*N,N'-[[(2-Aminoethyl)imino]di-3,1-propanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**74**)*

This was prepared in 56% yield from imidazolide **68** and *N,N'*-bis(2-aminoethyl)-1,3-propanediamine, as for **69**, as a pale orange solid, mp 130-131°C (from cetonitrile/chloroform). ^{1}H NMR ($CDCl_3$) δ 1.83 (m, 1 H, CCH_2C), 2.88 (t, 2 H, $NHCH_2$), 2.93 (t, 2 H, $NHCH_2$), 3.67 (q, 2 H, $CONHCH_2$), 7.27 (t), 7.45 (d), 7.51(t), 7.60 (t), 7.90 (d), 8.01 (d), 8.22 (s), 8.80 (d), 10.98 (s, 1 H, NH). Anal. ($C_{42}H_{34}N_6O_4.H_2O$) C, H, N.

*N,N'-[[(2-Aminoethyl)methylimino]di-2,1-ethanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**75**)*

This was prepared from imidazolide **68** and *N,N'*-bis(2-aminoethyl)-*N,N'*-dimethyl-1,2-ethanediamine, as for **69**. The first material obtained was dissolved in dichloromethane and addition of hexane gave the product in 70% yield. Column chromatography (alumina/chloroform) followed by recrystallization from acetonitrile gave a

sample with mp 192-193°C. ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3 H, NCH_3), 2.69 (t, 2 H, NCH_2), 2.77 (s, 2 H, NCH_2), 3.59 (q, 2 H, CH_2NH), 7.30 (t), 7.47-7.51 (m, 2 H), 7.60 (t), 7.88 (d), 8.09 (d), 8.15 (s), 8.72 (d), 10.88 (s, 1 H, NH). ESMS: m/z 689 (M+1). Anal. ($C_{42}H_{36}N_6O_4 . 0.5\ H_2O$) C, H, N.

*N,N′-[[(2-Aminoethyl)methylimino]di-3,1-propanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**76**)*

This was prepared in 75% yield from imidazolide **68** and *N,N′*-bis(2-aminoethyl)-*N,N′*-dimethyl-1,3-propanediamine, as for **69**. Further purification was by way of a perchlorate salt, prepared in EtOH and recrystallized from ethylene glycol. The free base was liberated, mp 107-110°C, which still contained trace impurities. ^{1}H NMR ($CDCl_3$) δ 1.80-2.0 (m, 1 H, CCH_2C), 2.34 (s, 3 H, NCH_3), 2.50-2.60 (m, 4 H, NCH_2), 3.55-3.65 (m, 2 H, CH_2NH), 7.18-7.24 (m, 2 H), 7.42 (t), 7.62 (t), 7.89 (d), 8.06 (d), 8.11 (s), 8.81 (d), 10.78 (br s, NH). ESMS: *m/z* 703.3 (95%) (M + 1); 352.2 (100%) [(M + 2)/2].

*N-[3-[[4-(11-oxo-11H-indeno[1,2-b]quinoline-6-carbonylamino)butyl]amino]propyl]-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**77**).*

This was prepared in 69% yield from imidazolide **68** and spermidine, as for **69**, and had mp 186-188°C (from $CHCl_3$/MeCN). ^{1}H NMR ($CDCl_3$) d 1.5-2.1 (m, 6 H, C-CH_2), 2.80-3.0 (m, 4 H, NCH_2), 3.6-3.8 (m, 4 H, CH_2NHCO), 7.35-7.46 (m, 2 H), 7.55-7.70 (m, 6 H), 7.80-8.0 (m, 4 H), 8.30-8.35 (m, 2 H), 8.75-8.85 (m, 2 H), 10.8-11.0 (br s, 2 H, NH). ESMS: m/z 660.2 (100%) (M + 1).

*N,N′-[[(2-Aminoethyl)imino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**78**)*

This was prepared in 69% yield from **42g** and N,N'-bis(2-aminoethyl)-1,3-propanediamine (2:1 mol ratio), as for **12g**. The crude product was subjected to column

chromatography (alumina; $CHCl_3$/MeOH, 96:4) and the fraction with R_f = 0.55 was collected. The solvent was removed under reduced pressure to give the product, mp 138-140°C (from EtOH). ^{1}H NMR ($CDCl_3$) d 1.60-1.8 (m, 1 H, C-CH_2), 2.7-2.8 (m, 5 H, CH_3+NCH_2), 2.85-2.95 (m, 2 H, NCH_2), 3.60-3.70 (m, 2 H, CH_2NHCO), 7.25-7.40 (m, 2 H), 7.50-7.55 (m, 2 H), 7.85 (d), 8.27 (s), 8.75 (d), 10.69 (br s, 1 H, NH). ESMS: m/z 703 (M + 1), 352 [(M + 2)/2]. Anal. ($C_{43}H_{38}N_6O_4.3H_2O$): C, H, N.

*N,N'-[[(2-Aminoethyl)methylimino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**79**).*

This was prepared in 57% yield from **42g** and N,N'-bis(2-aminoethyl)-N,N'-dimethyl-1,3-propanediamine (2:1 mol ratio), as for **78**. Column chromatography (alumina; $CHCl_3$/MeOH, 99:1) gave the product, R_f = 0.20, mp 103-105°C. ^{1}H NMR ($CDCl_3$) d 1.55-1.7 (m, 1 H, C-CH_2), 2.24 (s, 3 H, NCH_3), 2.40-2.45 (m, 2 H, NCH_2), 2.54-2.60 (m, 2 H, NCH_2), 2.76 (s, 3 H, CH_3), 3.54-3.65 (m, 2 H, CH_2NHCO), 7.25-7.35 (m, 2 H), 7.48-7.60 (m, 2 H), 7.86 (d), 8.24 (s), 8.75 (d), 10.58 (br s, 1 H, NH). ESMS: m/z 731.2 (40%) (M + 1); 366.4 (80%) [(M + 2)/2].

*N,N'-[[(3-Aminopropyl)imino]di-3,1-propanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] (**80**)*

This was prepared from imidazolide 68 and N,N'-bis(3-aminopropyl)-1,3-propanediamine, as for **69**. The crude product was extracted with hot light petroleum (bp 90-110°C), then with MeCN to give a pale orange solid (78%), mp >300°C. ^{1}H NMR (DMSO/TFA) d aliphatic region poorly resolved, 7.58 (t), 7.70-7.75 (m, 2 H), 7.92 (d), 8.04 (d), 8.48 (d), 8.53 (s), 8.62 (d), 10.59 (br s, NH). ESMS: 703 (M+1).

N,N′-[[(2-Aminoethyl)methylimino]di-2,1-propanediyl]bis-[6-oxo-6H-indeno[2,1-b]quinoline-4-carboxamide] ***(81)***

This was prepared in 33% yield from 47 and N,N′-bis(2-aminoethyl)-N,N′-dimethyl-1,3-propanediamine, after the crude product was extracted with hot light petroleum (bp 90-110°C) (x 2), then with hot MeCN, and obtained as a yellow solid, mp 207-209°C (from EtOH/$CHCl_3$). ^{1}H NMR d 1.79 (m, 1 H, CH_2), 2.35 (s, 3 H, NCH_3), 2.57 (t, 2 H, NCH_2), 2.70 (t, 2 H, NCH_2), 3.64 (m, 2 H, CH_2NHCO), 7.24 (t), 7.44-7.58 (m, 4 H), 7.73 (d), 7.97 (s), 8.58 (d), 10.60 (br s, NH). ESMS: 703 (M+1), 352 [(M+2)/2].

Example 8 Preparation of Further Carboxamides

N-[2-(Dimethylamino)ethyl]-10H-quindoline-4-carboxamide (7)

10*H*-Quindoline-4-carboxylic acid **38** (0.22 g) in thionyl chloride (3 mL) was heated at 80°C for 1 h and the excess thionyl chloride was removed at 20 mmHg. The residue was washed by decantation with dry CH_2Cl_2 (2 x 3 mL), and fresh CH_2Cl_2 (3 mL) was added. The mixture was cooled to 0°C, and stirred, and *N,N*-dimethylethylene-1,2-diamine (0.10 g) was added. After being stirred at room temperature for 1 h, the solution was filtered and the filtrate washed with 10% Na_2CO_3 solution, water, dried ($MgSO_4$) and the solvent removed to give 7 (0.17 g, 63%), mp 251-254°C (from MeCN), which could not be freed from a trace impurity. ^{1}H NMR δ 7.06 (t), 7.20-7.32 (m, 2 H), 7.41 (t), 7.73 (d), 7.80 (s), 8.14(d), 8.64 (d), 9.72 (s, ring NH), 11.92 (br t, amide NH).

N-[2-(Dimethylamino)ethyl]-8-chlorobenzofuro[2,3-b]-quinoxaline-10-carboxamide ***(15)*** *and N-[2-(Dimethylamino)-ethyl]-9-chlorobenzofuro[2,3-b]quinoxaline-7-carboxamide* ***(23)***

The precursor isomeric acid mixture was reacted with thionyl chloride and then *N,N*-dimethylethylene-1,2-diamine as for the preparation of **7**. The crude product was first washed through a short alumina column with $CHCl_3$

and the solvent removed to give the carboxamide mixture as a yellow solid (56%). This (0.18 g) was recrystallized from MeCN to give a sample of 23 (containing <10% 15 by NMR) (0.04 g), mp 205-212°C. ^{1}H NMR ($CDCl_3$) δ 7.54 (t), 7.70 (d), 7.75 (t), 8.24 (s), 8.34 (d), 8.80 (s), 10.82 (s, NH). The recrystallization filtrate was evaporated to dryness and the residue was stirred with cold MeCN (2 x 2 mL) and filtered each time. Evaporation of the solvent from the combined filtrates gave **15** (containing <5% 23 by NMR) (0.07 g), mp 128-133°C. ^{1}H NMR ($CDCl_3$) δ 7.53 (t), 7.69 (d), 7.77 (t), 8.32 (d), 8.37 (s), 8.80 (s), 10.19 (s, NH). Alkaline hydrolysis gave the corresponding carboxylic acid. Anal. (on a sample of the isomeric amide mixture, recrystallized from MeCN) ($C_{19}H_{17}ClN_4O_2$) H, N; C: calc, 61.9; found, 61.4.

*N,N'-Bis[2-(Dimethylamino)ethyl]-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxamide (**10b**).*

This was prepared as for (**7**) as a yellow solid (63%), mp 193-195°C (recrystallized twice from MeCN and once from EtOH). ^{1}H NMR ($CDCl_3$) d 2.27-2.30 (m, 12 H, NCH_3), 2.58 (t, 2 H, CH_2N), 2.65(t, 2 H, CH_2N), 3.65 (q, 2 H, CH_2NH), 3.74 (q, 2 H, CH_2NH), 7.08 (br s, 1 H, NH), 7.55-7.65 (m, 2 H), 7.65 (t), 7.81 (d), 8.10 (d), 8.34 (d), 8.78 (d), 11.03 (br s, NH). Anal. ($C_{26}H_{29}N_5O_3.H_2O$): C, H, N.

*N,N'-Bis[2-(Dimethylamino)ethyl]-4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6,10-dicarboxamide (**10c**).*

This was prepared as for (**7**) as a tan solid (66%), mp 204-206°C (from MeCN). ^{1}H NMR ($CDCl_3$) d 2.25-2.30 (m, 12 H, NCH_3), 2.54 (t, 2 H, CH_2N); 2.67 (t, 2 H, CH_2N), 2.82 (s, 3 H, CH_3), 3.60 (q, 2 H, CH_2NH), 3.72 (q, 2 H, CH_2NH), 7.1 (br s, 1 H, NH), 7.38-7.50 (m, 2 H), 7.61 (t), 7.68 (d), 8.10 (d), 8.76 (d), 10.5 (s, 1 H, NH). Anal. ($C_{27}H_{31}N_5O_3.H_2O$): C, N; H calcd, 6.8; found: 6.1.

*N-[2-(Dimethylamino)ethyl]-4-methoxy-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide (**12e**)*

A solution of ester **36** (0.4 g, 1.2 mmol) and *N,N*-dimethylethylenediamine (0.6 g, 6.8 mmol) in anhydrous 1-propanol (16 mL) was stirred and heated at reflux for 2 days under an atmosphere of nitrogen. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL), then washed with 10% $NaHCO_3$ (3 x 50 mL), warm water (2 x 50 mL) and dried ($MgSO_4$). The CH_2Cl_2 was removed under reduced pressure and the residue was subjected to column chromatography (alumina/$CHCl_3$), with the fraction R_f = 0.3 being collected. The solvent was removed under reduced pressure and the residue was recrystallized from MeCN to give the product as a yellow solid (50 mg, 11%), mp 195-197°C. ^1H NMR ($CDCl_3$) δ 4.14 (s, 3 H, OCH_3), 7.25 (d), 7.44-7.56 (m, 2 H), 7.62 (t), 7.95 (d), 8.37 (s), 8.86 (d), 11.58 (s, 1 H, NH). Anal. ($C_{22}H_{21}N_3O_3.H_2O$) C, H, N.

*11,11'-(O,O'-Hexane-1,6-diylbisisonitroso)bis-[N-[2-(dimethylamino)ethyl]-11H-indeno[1,2-b]quinoline-6-carboxamide (**73**)*

A solution of amide **12a** (0.4 g) and O,O'-1,6-hexanediylbishydroxylamine dihydrochloride (0.12 g) in 5% hydrochloric acid (10 ml) was heated under reflux for 3h. The solution was cooled to room temperature., and basified to Ph 10 with 10% sodium hydroxide. The oily reside which formed was extracted into chloroform, washed with water (2 x 10 ml) and dried over $MgSO_4$. Removal of the solvent gave the product as an orange oil (0.39 g, 84%). ^1H NMR complex and poorly resolved. ESMS: m/z 803 (M+1), 402 ((M+2)/2). A hygroscopic perchlorate salt was prepared in isopropanol and dried in vacuo, and had mp 167-169°C. Anal. ($C_{48}H_{50}N_8O_4.2HClO_4.4H_2O$) C, H, N.

Example 9 Anti-Tumour Activity *In Vitro*

The two series of tetracyclic quinoline and quinoxaline carboxamides, identified in Table 1, were all evaluated for growth inhibitory properties, measured as IC_{50} values for continuous *in vitro* drug exposure for 72 h against the murine leukaemia P388 and the late-passage murine Lewis lung carcinoma line LLTC, as examples of murine leukaemias and carcinomas. They were also assessed against a wild-type human leukemia line (Jurkat; JL_C), and two sublines (JL_A and JL_D). These lines have previously been described in detail (Finlay *et al*, 1994; Finlay *et al*, 1996). The JL_A line is resistant to the DNA intercalator amsacrine and similar agents by virtue of a reduced level of topo II enzyme. The JL_D line is resistant to doxorubicin, primarily by virtue of altered levels of topo II, but probably also by additional mechanisms.

The ratios of the IC_{50} values of a drug in the parent line compared with one of the sublines ($IC_{50}[JL_A]/IC_{50}[JL_C]$ and ($IC_{50}[JL_D]/IC_{50}[JL_C]$) therefore provide some indication of the mechanism of cytotoxicity. Classical topo II inhibitors such as amsacrine, doxorubicin and etoposide have large ratios (10-90 fold), whereas topo I inhibitors such as camptothecin and mixed topo I/II inhibitors such as DACA (**4**) have ratios of only about 2-fold (Table 2). Values of these ratios of less than about 1.5-2 therefore suggest cytotoxicity by a non-topo II mediated mechanism.

Murine P388 leukemia cells, Lewis lung carcinoma cells (LLTC), and human Jurkat leukemia cells (JL_C), together with their amsacrine- and doxorubicin-resistant derivatives (JL_A and JL_D respectively), were obtained and cultured as previously described (Finlay *et al*, 1994; Finlay *et al*, 1996). Growth inhibition assays were performed by culturing cells at 4.5×10^3 (P388), 10^3 (LLTC), and 3.75×10^3 (Jurkat lines) cells per well in microculture plates (150 μl per well) for 3 (P388) or 4 days in the presence of drug. Cell growth was determined by [^{3}H]-thymidine uptake (P388)

(Marshall *et al*, 1992) or the sulphorhodamine assay (Skehan *et al*, 1990). Independent assays were performed in duplicate, and coefficients of variation were 12% (P388) 12% (LLTC), 6.3% (JL_C), 9.3% (JL_A), and 5.7% (JL_D). The results are summarized in Table 5.

Table 5

Anti-Tumour Activity *In Vitro* of Tetracyclic Quinoline and Quinoxaline Carboxamides

Compound	IC_{50} (nM) [a]			IC_{50} ratios	
	P388 [b]	LLTC [c]	JL_C [d]	JL_A/JL_C	JL_D/JL_C
7	370	290	450	1.00	1.07
8	170	210	430	1.16	1.24
9	46	66	170	1.46	1.61
10	25	30	34	0.52	0.86
11	88	190	320	1.76	1.89
12a	130	91	180	1.24	0.89
12b	43	34	106	0.65	0.7
12c	438	58	68	1.1	1.2
12d	134	76	102	1.0	1.0
12e	23	23	71	2.2	0.8
12f	62	59	106	1.1	1.2
12g	13.5	15	35	2.1	0.9
12j	143	17	42	0.8	0.9
12k	135	152	325	1.3	1.5
13	1800	430	860	3.16	4.25
14	1600	700	980	0.77	0.91
15	5800	>2000	>2000	Nd [f]	Nd [f]
16	360	390	550	0.97	0.96
17	700	880	1100	1.04	1.07
18	100	110	150	1.90	2.75
19	85	150	370	0.88	0.94
20	670	990	1400	0.86	0.98
21	15000	360	300	1.23	1.29
22	2200	1700	2600	1.00	1.00

23	6800	1400	1500	1.19	>1.30
69	1188	240	165	0.4	0.7
70	34	14	2.5	0.2	0.5
71		12	23	0.35	0.5
72		2.4	2.3	0.3	0.4
73	925	680	1100	0.8	0.5
74	26	4.7	0.75	0.3	0.5
75	520	33	21	0.3	0.5
76	18	2.7	0.35	0.3	0.4
DACA	71	190	580	1.87	2.29
amsacrine	20	12	37	84.9	73.7
doxorubicin	15	22	9.6	4.39	12.7
etoposide	25	180	160	13.3	90.3
camptothecin	-	33	5.6	1.95	1.40

[a] IC_{50}; concentration of drug to reduce cell number to 50% of control cultures (see text)

[b] Murine P388 leukaemia

[c] Murine Lewis lung carcinoma

[d] Human Jurkat leukaemia

[f] Not determinable (both IC_{50}s>2000 nM)

na not applicable

The monocationic quinoline analogues **7-9**, **11** and **12a** showed broadly similar cytotoxicities to DACA (IC_{50}s of 46-370 nM against P388, 66-290 nM against Lewis lung, and 170-450 nM against the wild-type human line JL_C). This compares with IC_{50}s of 98, 200 and 580 nM respectively for DACA. The thieno and indeno analogues **9** and **12a** were the most active, being about 2-fold more cytotoxic than DACA against JL_C. The dicationic thieno derivative **10** was more cytotoxic again (JL_C IC_{50} 34 nM). All of these compounds had low JL_A/JL_C and JL_D/JL_C ratios, suggesting that this cytotoxicity does not result primarily from inhibition of

topo II. The resistance properties of these compounds resemble those of 7-chloroDACA, which has been shown to stimulate sequence-selective cleavage of DNA in response to topo I (Finlay *et al*, 1996). In contrast, the thienodioxide analogue **13** was much less cytotoxic, possibly because of a less coplanar ring system which could compromise intercalative binding, and had larger IC_{50} ratios of about 3.

The quinoxaline analogues **14-18** had IC_{50} values which varied considerably (*eg.* from 130 to 1500 nM against JL_C), but were on average somewhat less cytotoxic than the quinolines. However, their IC_{50} ratios against the Jurkat cell lines were also generally around unity, suggesting a similar mode of action to the quinoline derivatives. The effect of the chloro group in **14** and **15** could not be determined precisely, since the corresponding unsubstituted derivatives were not available, but the IC_{50} results suggest that it is probably not advantageous.

In the isomeric quinoline series, only the indeno analogue (**19**) was available, and this compound showed a pattern of activity comparable to its isomer **12a**. The isomeric quinoxalines (**20-23**) were found to be less cytotoxic on average than their counterparts. In this series there were two sets of compounds (**20/21** and **22/23**) available to evaluate the effects of a chloro substituent, and an analysis of these results suggests that it does increase potency, at least against the human cell lines. This position might therefore be used to manipulate other physicochemical aspects, such as lipophilicity, which might affect pharmacology and metabolism.

The *bis* compounds **69-76** showed particularly striking activity, with IC_{50} values in some cases far lower than for DACA, and comparable to or lower than for the established anti-cancer agent doxorubicin.

Example 10 Anti-Tumour Activity *In Vivo*

Compounds **10** and **12a** were evaluated against murine colon 38 tumours implanted subcutaneously in C57BL/6 mice. This advanced colon 38 tumor model is fairly refractory to standard clinical topo II agents, with doxorubicin and etoposide providing growth delays of 8 and 1.5 days respectively, using the administration schedules described below (Baguley *et al*, 1995).

Colon 38 tumours were grown subcutaneously from 1 mm^3 fragments implanted in one flank of mice which had been anaesthetised with pentobarbitone, 90 mg/kg. When tumours reached a diameter of approximately 4 mm (7-8 days), mice were divided into control and drug treatment groups (5 mice/group), with similar average tumour volumes in each group. Solutions **10** and **12a** in distilled water were injected intraperitoneally in a volume of 0.01 mL/g body weight, every fourth day for 3 treatments.

The mice were monitored closely, and tumour diameters were measured with callipers three times a week. Tumour volumes were calculated as $0.52 \times a^2 \times b$ mm^3, where a and b are the minor and major tumour axes, and the data were plotted on a semilogarithmic plot as mean tumour volumes (± SEM) versus time after treatment. The results are shown in Figures 7 and 8. The growth delay was calculated as the time taken for tumours to reach a mean volume four-fold higher than their pre-treatment volume.

Drug treatment with **10** and **12a** was initiated at a time when the tumours were approximately 4 mm in diameter. The maximum tolerated dose was 90 mg/kg/dose for compound **12a**, and 20 mg/kg/dose for compound **10**. Compound **12a** provided a growth delay of about 7 days, and compound **10** provided a growth delay of 5.3 days, as shown in Figure 7. By comparison, the mixed topo I/II inhibitor DACA provided a growth delay of 8.8 days for this administration schedule, and up to 22 days for extended schedules (Baguley *et al*, 1995).

DISCUSSION

Overall, this new class of compounds appears to have a mechanism of cytotoxicity similar to that of the acridine-4-carboxamide DACA, which is a mixed topo I/II inhibitor (Baguley *et al*, 1995). Some of the analogues, especially within the *bis* series, are many times more cytotoxic than DACA in the human leukaemia cell lines studied here, and the two derivatives (**10** and **12a**) so far evaluated *in vivo* show a growth delay which is comparable to that of the clinical topo II agent doxorubicin, and superior to that of etoposide. The results suggest that the compounds of the invention are a new class of mixed topo I/II inhibiting compounds, and are useful as anticancer drugs.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this invention.

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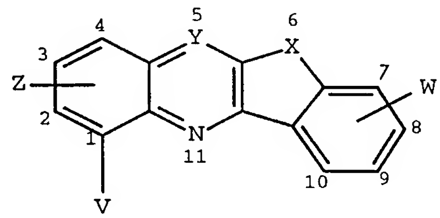
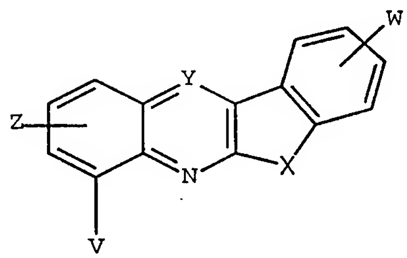
CLAIMS:

1. a compound of general formula I or general formula II



I



II

in which positional numbering, where mentioned, refers to the arbitary system illustrated for formula I above, and

in which V is $C(=U)NR(CH_2)_nR^1$, where U is O or S, R is hydrogen or a C_{1-4} alkyl group which is optionally substituted with one or more OH or NH_2 groups, and R^1 is $C(=NH)NH_2$, $NHC(=NH)NH_2$ or NR^2R^3, where each of R^2 and R^3 is independently hydrogen or a C_{1-4} alkyl group which is optionally substituted with one or more OH or NH_2 groups, and n is an integer from 1 to 6;

Y is CH, N or C-V

X is CH_2, CH-C_{1-4} alkyl, CO, O, S, SO, SO_2, N-C_{1-4} alkyl or NH;

Z is H, F, Cl, Br, I, OH, NR^2R^3, nitro, cyano, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy 2,3- or 3,4-methylenedioxy, or 3,4-ethylenedioxy; and

W is H, F, Cl, Br, I, OH, NR^2R^3, nitro, amino, cyano, benzo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6}

haloalkoxy, 7,8- 8,9- or 9,10-methylenedioxy or ethylenedioxy,

or a pharmaceutically-acceptable salt or N-oxide thereof.

2. A compound according to Claim 1, in which X is NH, CO, CH_2, O or S; Y is CH, N or C-CONH$(CH_2)_2$N$(CH_3)_2$; Z is H, methoxy or Cl; W is H, methoxy, methyl, Cl, hydroxy or benzo; and n is 2.

3. A compound according to Claim 1 or Claim 2, in which X is CH_2 or NH, of which a hydrogen is optionally substituted with a C_{1-4} alkyl group.

4. A compound according to any one of Claims 1 to 3, in which W is benzo, and is linked 7,8; 8,9; 9,10; or 6,6a,7.

5. A compound according to any one of Claims 1 to 4, in which R is hydrogen.

6. A compound according to any one of Claims 1 to 5, in which the compound is of general formula I, and X is CO, Y is CH, and Z is H.

7. N-[2-(dimethylamino)ethyl]-11-oxo-11*H*-indeno[1,2-*b*]-quinoline-6-carboxamide, or a pharmaceutically-acceptable salt or N-oxide thereof.

8. N-[2-(dimethylamino)-ethyl]-11-oxo-4-methyl-11H-indeno[1,2-b]quinoline-6-carboxamide,or a pharmaceutically acceptance salt or N-oxide thereof

9. A compound in which two units of general formula I or general formual II as defined in any one of Claims 1 to 6 respectively are linked via a symmetrical or non-symmetrical linker group, or a pharmaceutically-acceptable salt or N-oxide thereof.

10. A compound according to Claim 9, in which the linkage is through V, and the group NR$(CH_2)_n$R' is replaced in each subunit of the *bis* compound by a linker group selected from the group consisting of:

-NH$(CH_2)_3$NH$(CH_2)_4$NH-

-NH$(CH_2)_3$NH$(CH_2)_3$NH$(CH_2)_3$NH-

-NH$(CH_2)_2$NH$(CH_2)_2$NH-

$-NH(CH_2)_3-NMe-(CH_2)_3NH-$
$-NH(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH-$
$-NH(CH_2)_2NH(CH_2)_3NH(CH_2)_2NH-$
$-NH(CH_2)_2NMe(CH_2)_2NMe(CH_2)_2NH-$
$-NH(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2NH-$
-N,N'-Bis(2-aminoethyl)piperazine-
N,N'-Bis(3-aminopropyl)piperazine,
where Me represents methyl.

11. A compound according to Claim 10, selected from the group consisting of N,N'-[[(2-Aminoethyl)imino]di-3,1-propanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide], N,N'-[[(2-aminoethyl)methylimino]di-3,1-propanediyl]bis-[11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide], N,N'-[[(2-aminoethyl)imino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide] and N,N'-[[(2-aminoethyl)-methylimino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11H-indeno[1,2-b]quinoline-6-carboxamide], or a pharmaceutically-acceptable salt or N-oxide thereof.

12. A compound according to Claim 9 in which the linkage is through X, the H of CH_2 or NH is replaced in each subunit by a link via $-(CH_2)_m$, where m is an integer from 1 to 12; O of C=O is replaced in each subunit by a bis-olefinic link via $=CH(CH_2)_n-CH=$; or O of C=O is replaced in each subunit by a bis-oxime link via $=N-O-(CH_2)_p-O-N=$, where p is an integer from 1 to 8.

13. A compound according to Claim 9 in which the linker is of the form $-NH(CH_2)_mNH(CH_2)_nNH-$ or $-NH(CH_2)_mNAlkyl(CH_2)_nNH$ or $-NH(CH_2)_mNH(CH_2)_nNH(CH_2)_oNH-$ or $-NH(CH_2)_mNAlkyl(CH_2)_nNAlkyl(CH_2)_oNH-$, where m, n and o are integers from 2 to 6.

14. A pharmaceutical composition comprising a compound of general formula I or general formula II according to any one of Claims 1 to 8 or a *bis* compound according to any one of Claims 9 to 13, together with a pharmaceutically-acceptable carrier.

15. A method of treatment of a neoplastic condition, comprising the step of administering an anti-tumour effective dose of a compound according to any one of Claims 1 to 13 to a mammal in need of such treatment.

16. A method according to Claim 15, in which the compound is administered simultaneously or sequentially with one or more other anti-neoplastic agents.

17. A method according to Claim 16, in which the other anti-neoplastic agent is an anti-mitotic agent, an anti-metabolite, a hormonal regulator, a DNA-reactive agent, or a biological agent.

18. A method according to Claim 16 in which the other anti-neoplastic agent is a DNA-binding anti-cancer agent.

19. A method according to any one of Claims 15 to 18 in which the compound is administered in a divided dose schedule, such that there are at least two administrations in total in the schedule.

20. A method according to Claim 19 in which the administrations are given at least every two hours for up to four hours or longer.

21. A method according to Claim 19 or Claim 20, in which the divided-dose schedule comprises a second administration of the compound of the invention after an interval from the first administration sufficiently long that the level of active compound in the blood has decreased to approximately from 5-30% of the maximum plasma level reached after the first administration, so as to maintain an effective content of active agent in the blood.

22. A method according to Claim 21, in which one or more subsequent administrations is given at a corresponding interval from each preceding administration, preferably when the plasma level has decreased to approximately from 10-50% of the immediately-preceding maximum.

23. A method according to any one of Claims 15 to 22 for treatment of leukaemias, lymphomas, sarcomas, or brain tumours, or for treatment of cancer of the lung, breast, ovary, testis, or colon.

24. A method of synthesis of a compound according to Claim 1 to 13 comprising the step of reacting an intermediate compound according to general formula I or general formula II in which V is COOH with an appropriate amino or diamino compound.

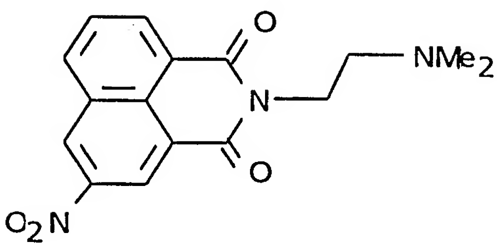
25. A method according to Claim 24 comprising one or more steps illustrated in any one of reaction schemes 1a, 1b, 1c, 2, 3a, 3b, 4a or 4b.

26. A method according to Claim 24 or Claim 25 in which the intermediate compound is a quinoline carboxylic acid or a quinoline dicarboxylic acid as described in Examples 1 to 3.

27. A method according to Claim 24 or Claim 25 in which the intermediate is a quinoxaline carboxylic acid.

28. A quinoline carboxylic acid or dicarboxylic acid as described in Example 2 or Example 3.

29. A quinoxaline carboxylic acid as described in Example 4, other than 11-oxo-11-H-indeno[1,2b]quinoxaline-6-carboxylic acid.



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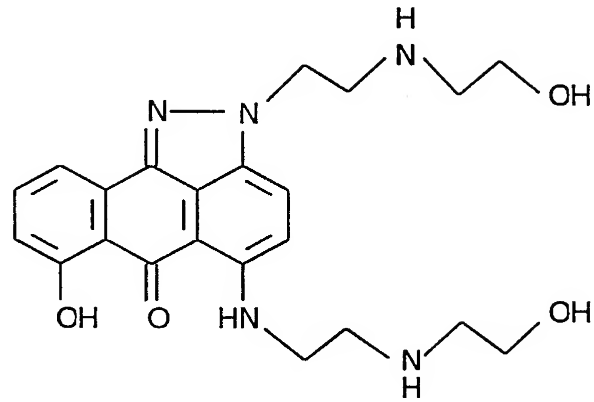

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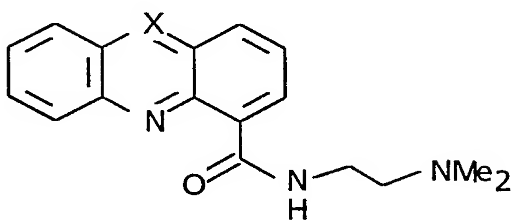
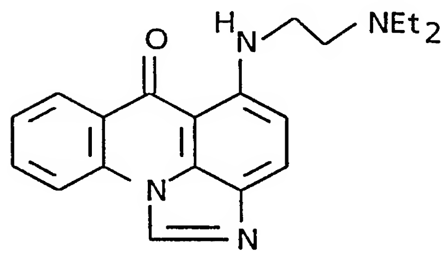


3 : X = N
4 : X = CH (DACA)



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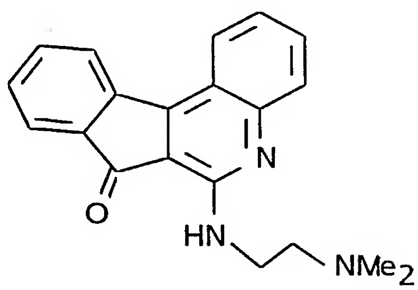

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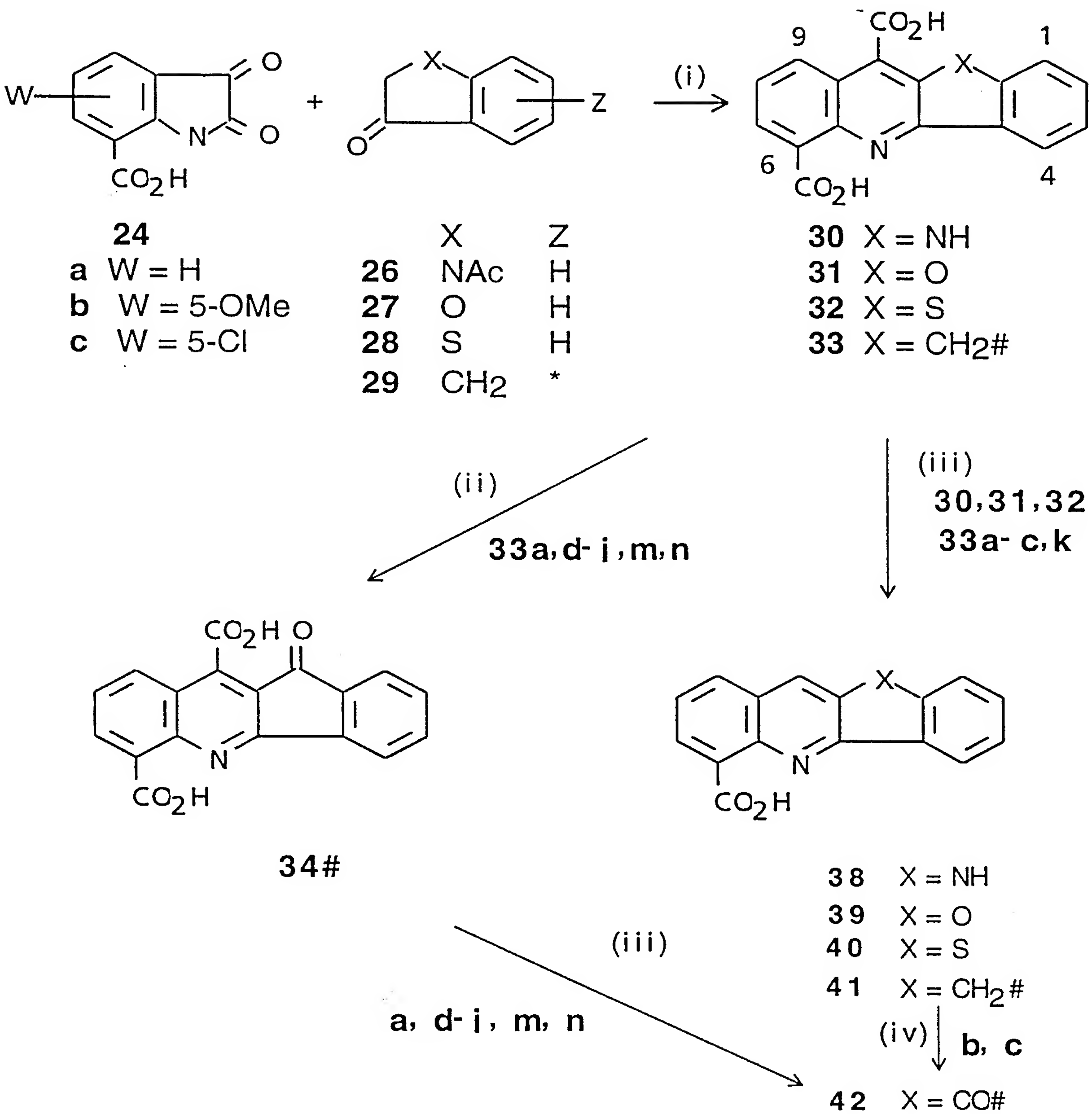
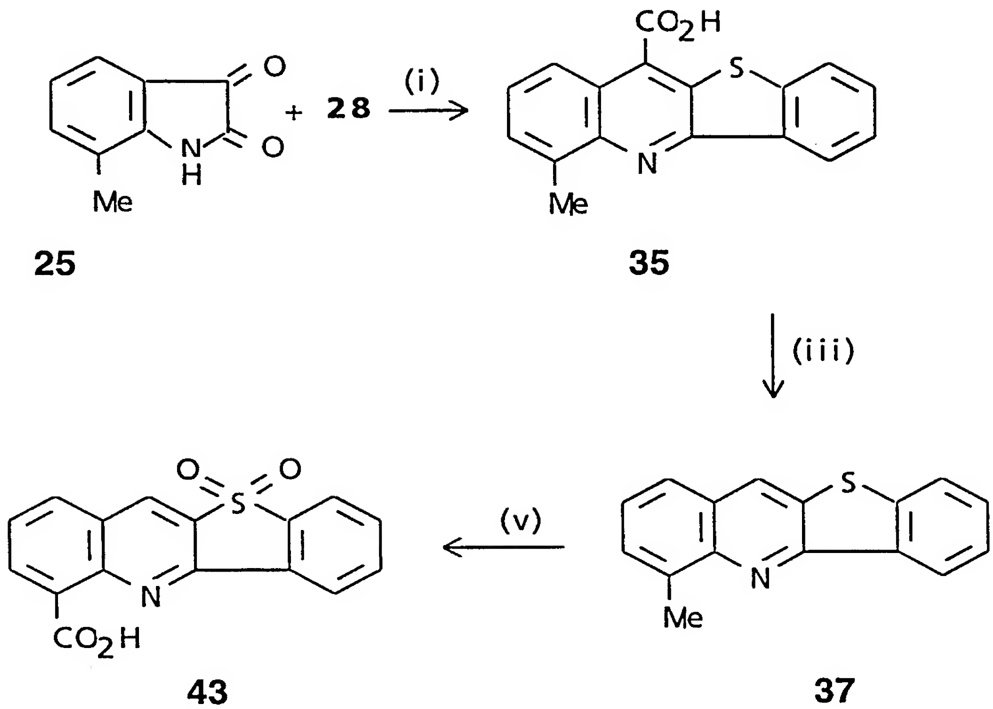
FIGURE 1

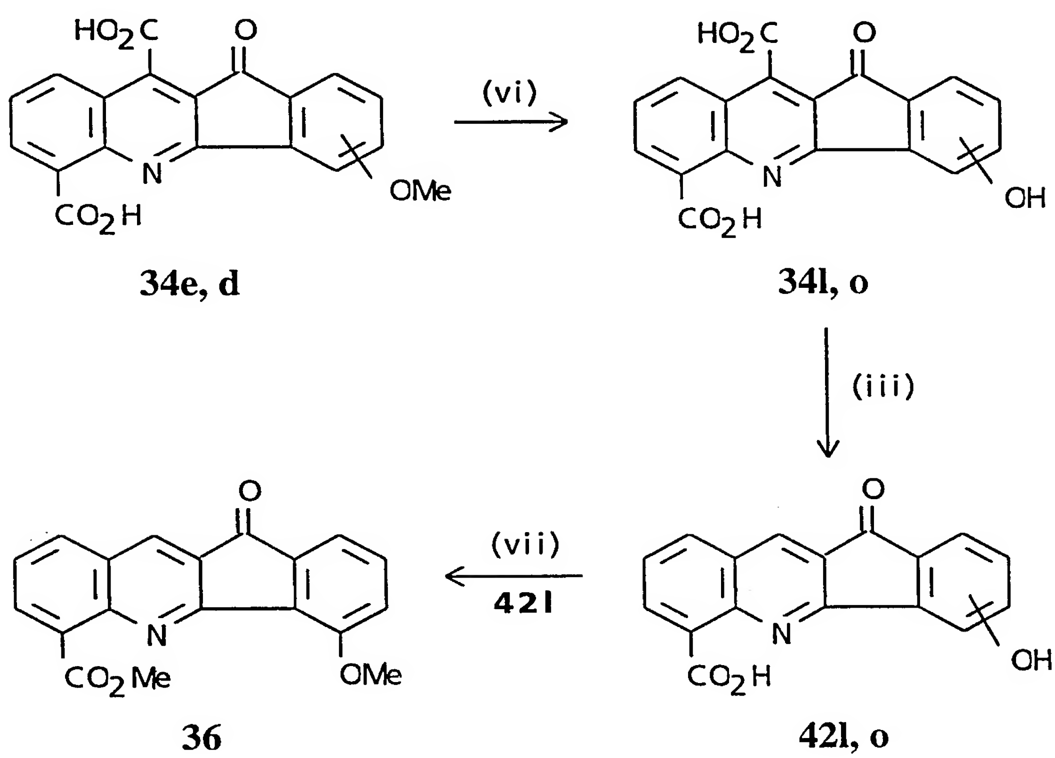


Scheme 1a

FIGURE 2



Scheme 1b



Scheme 1c

Figure 2 (cont.)

(i) $OH^-/H_2O/90$ °C (ii) $KMnO_4/Na_2CO_3/55$ °C (iii) heat *ca* 300 °C
(iv) $Na_2Cr_2O_7$/HOAc/reflux (v) CrO_3/H_2SO_4/HOAc/20 °C
(vi) $AlCl_3$/NaCl/180 °C (vii) MeI/Ag_2O/DMF/20 °C

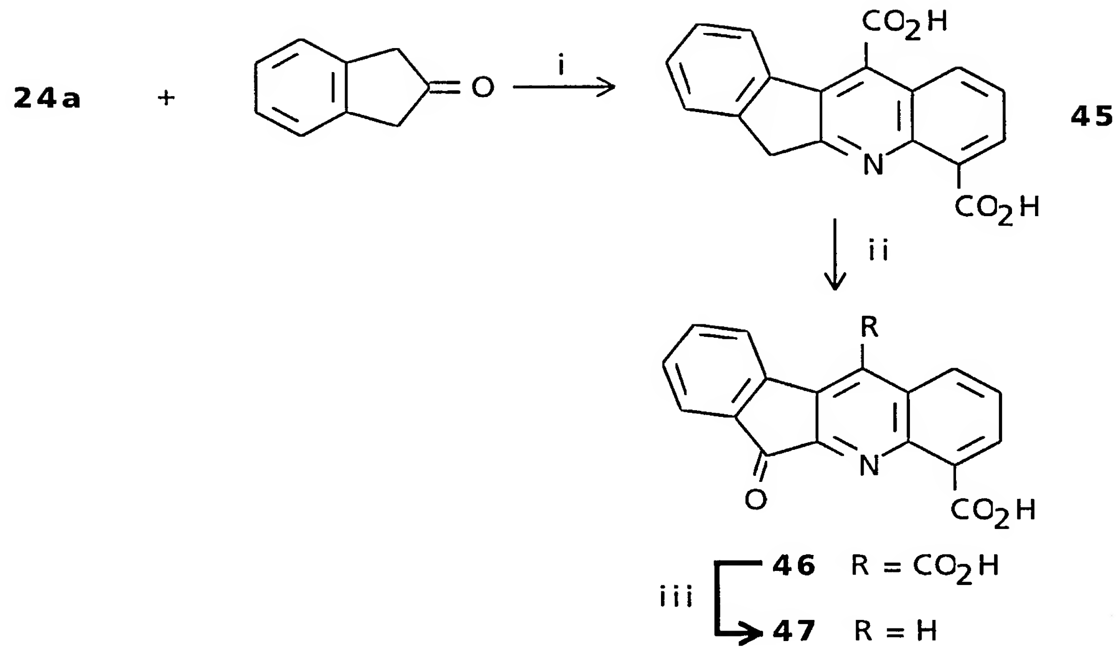
For compounds **29**:

	Z
a	H
b	4-OMe
c	5-OMe
d	6-OMe
e	7-OMe
f	6-Me
g	7-Me
h	5-Cl
i	7-Cl
j	5,6-$(OMe)_2$
k	3,3a,4-benzo

For compounds **33, 34, 41, 42,** (and amides**12**):

a	H
b	1-OMe
c	2-OMe
d	3-OMe
e	4-OMe
f	3-Me
g	4-Me
h	2-Cl
i	4-Cl
j	2,3-$(OMe)_2$
k	1,11a,11-benzo
l	4-OH
m	8-OMe
n	8-Cl
o	3-OH

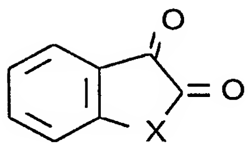
Figure 2(cont.)

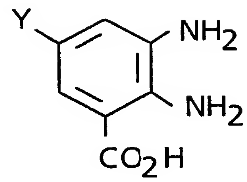


Scheme 2

i :$OH^-/H_2O/90$ °C
ii :$KMnO_4/Na_2CO_3/55$ °C
iii : heat *ca* 300 °C

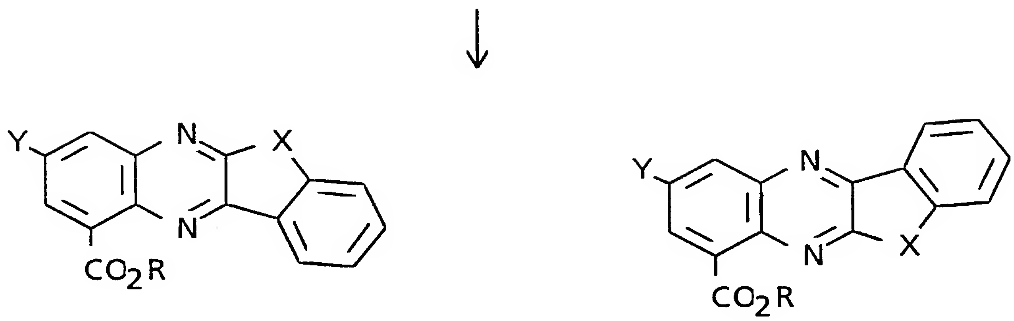
Figure 3





(**53**) X = O
(**54**) X = S
(**55**) X = NH

(**48**) Y = H
(**52**) Y = Cl

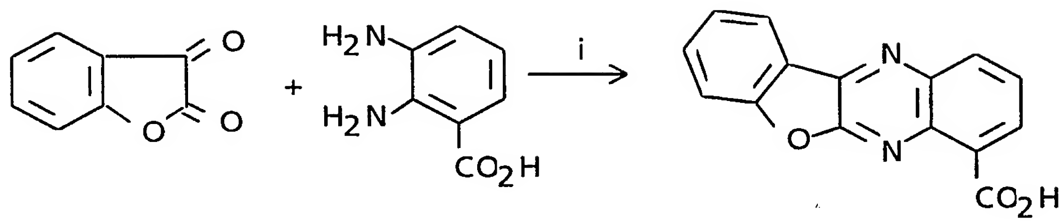


(Form A) (Form B)

	X	Y	R
49	O	H	H
59	O	Cl	H
62	NH	H	H
63	NH	H	Et
64	NH	Cl	H
65	NH	Cl	Et
66	S	H	H

Scheme 3a

FIGURE 4a

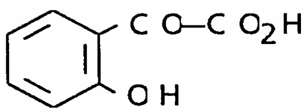


53 **48** **49b**

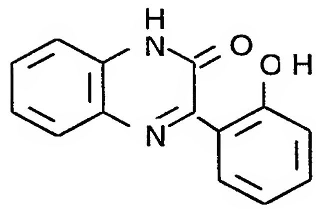
Scheme 3b

i : PPA/110 °C/5 h

Figure 4b



(56)



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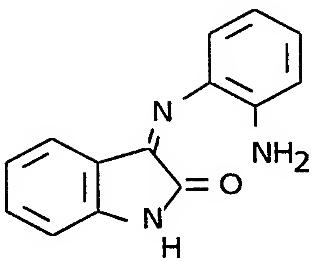

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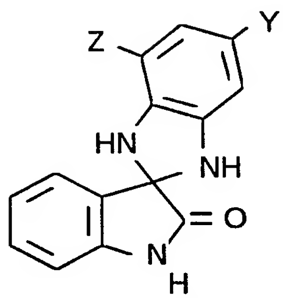
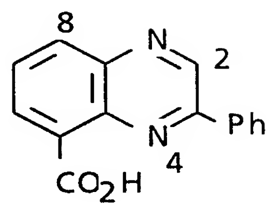


(61) Y = Z = H



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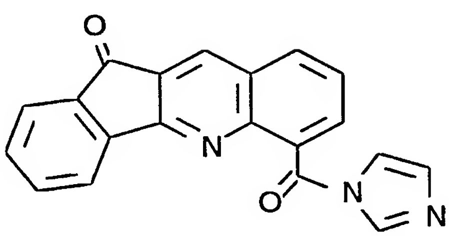
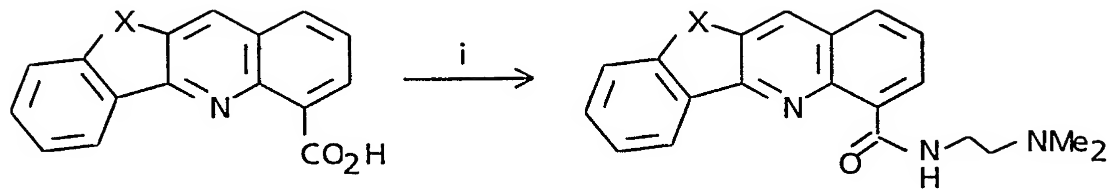


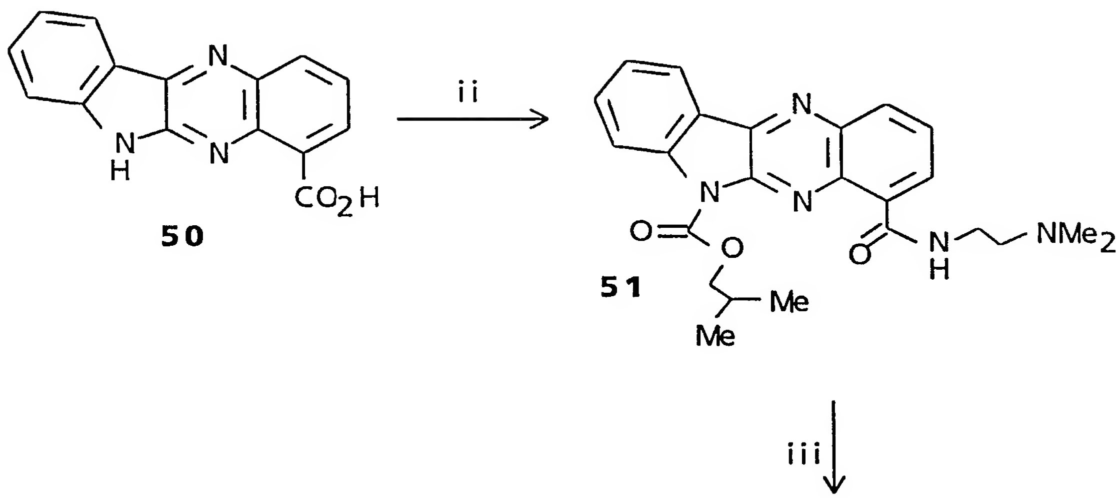
(68)

FIGURE 5



38-43, 47 compounds of Table 1

Scheme 4a



compound **20** of Table 1

Scheme 4b

i : Isobutyl chloroformate (1.2 equiv.)/CH_2Cl_2/NEt_3/1.5 h, or
1,1'-carbonyldiimidazole/dioxan/boil, or
thionyl chloride/80 °C/1 h, then
N,N-dimethylethylenediamine/CH_2Cl_2/0-20 °C

ii : As for (i) 2.4 equiv. of isobutyl chloroformate

iii : Aqueous NaOH/dioxan/20 °C/16 h.

FIGURE 6

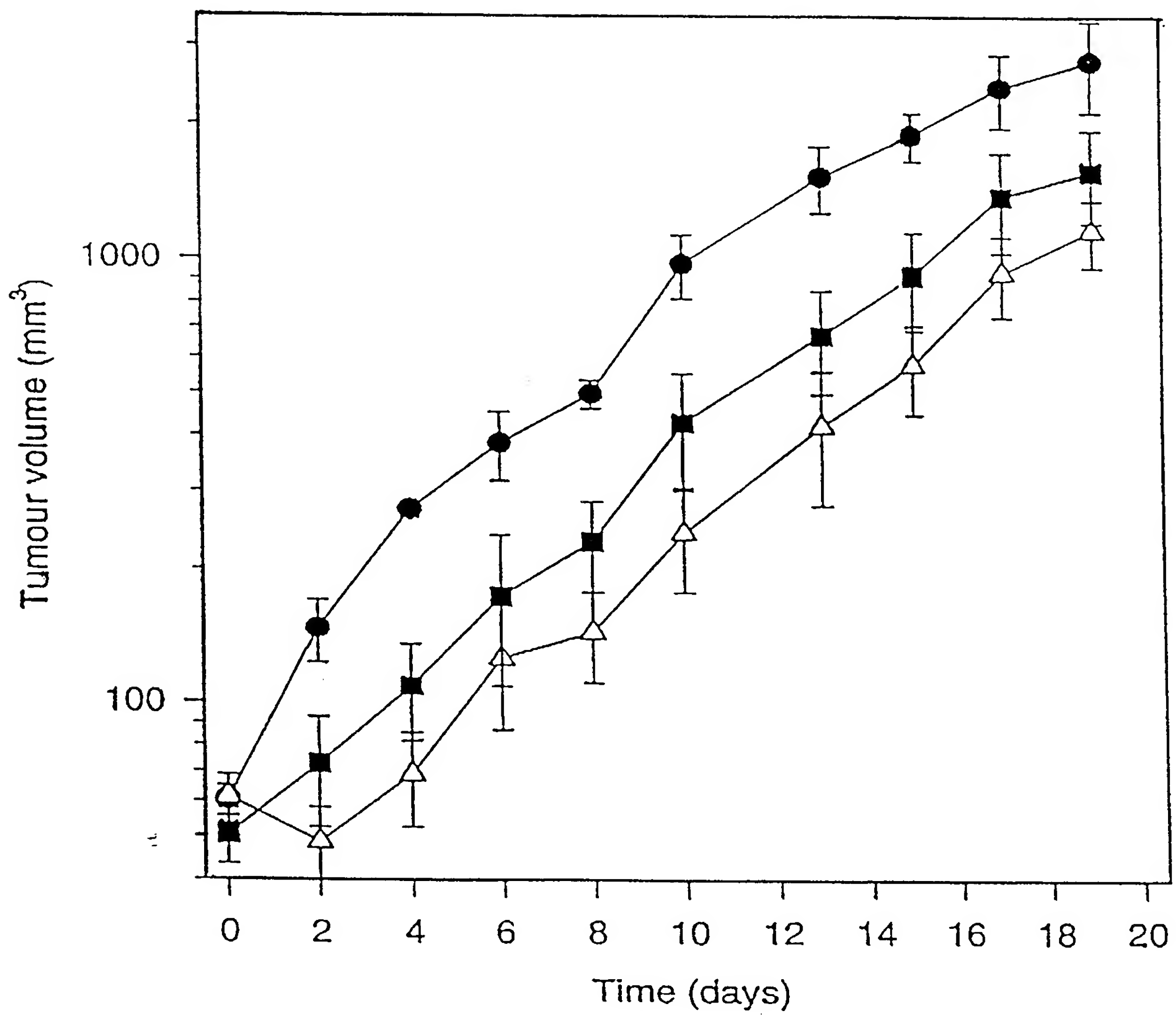


Figure 7

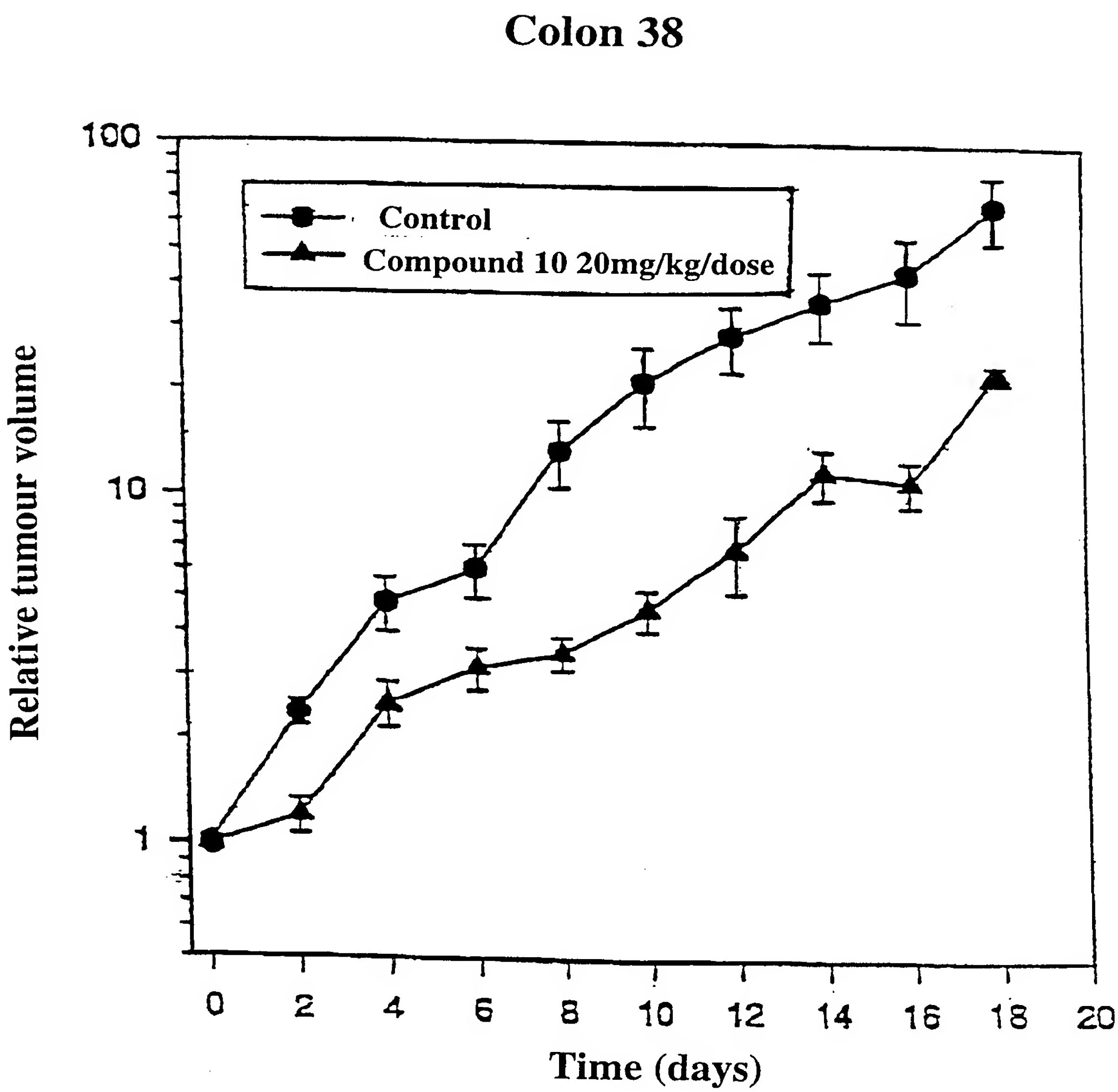

Colon 38
Control
Compound 10 20mg/kg/dose
Relative tumour volume
100
10
1
0
2
4
6
8
10
12
14
16
18
20
Time (days)

Figure 8

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00218

A. CLASSIFICATION OF SUBJECT MATTER	
Int Cl^{6}:	C07D 221/18, 495/04, 471/04, 491/048, 487/04, 519/00; A61K 31/47
According to International Patent Classification (IPC) or to both national classification and IPC	
B. FIELDS SEARCHED	
Minimum documentation searched (classification system followed by classification symbols)	
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched	
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN CAS ON-LINE SUBSTRUCTURE SEARCH	

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Deady, Leslie W; Kaye, Anthony J; Finlay, Graeme J; Baguley, Bruce C; Denny, William A; "Synthesis and Antitumour Properties of N-[2-(Dimethylamino)ethyl] carboxamide Derivatives of Fused Tetracyclic Quinolines and Quinoxalines: A New Class of Putative Topoisomerase Inhibitors", J Med Chem (1997), 40(13), 2040-2046 See whole document, particularly column 2 page 2040 to page 2042	1-29
A	Haldane, Andrea; Finlay, Graeme J; Baguley, Bruce C ; "A comparison of the effects of aphidicolin and other inhibitors on topoisomerase II-directed cytotoxic drugs", Oncol Res (1993), 5(3), 133-8 See page 133 column 1-2 to page 134 column 1-2	1,15

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search 29 April 1998	Date of mailing of the international search report -2 MAY 1998
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer **S.R. IDRUS** Telephone No.: (02) 6283 2536

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chen, Mei; Beck, William ; "Teniposide-resistant CEM cells, which express mutant DNA topoisomerase II alpha, when treated with non-complex-stabilising inhibitors of the enzyme, display no cross-resistance and reveal aberrant functions of the mutant enzyme", Cancer Res (1993), 53(24), 5946-53 See page 5947 column 1, page 5946 column 1-2	1, 15
A	Kusumoto, Hiroki; Rodgers, Queen E; Boege, Friedrich; Raimondi, Susana; Beck, William T; "Characterisation of novel human leikemic cell lines selected for resistance to merbarone, a catalytic inhibitor of DNA topoisomerase II", Cancer Res (1996), 56(11), 2573-2583 See page 2573 column 2 lines 1-8; 26-33 and page 2574 column 1 lines 24-33	1, 15
A	Wilson, W R; Denny, W A; Pullen, S M; Thompson, K M; Li, A E; Patterson, L H; Lee, H H; "Tertiary amine N-oxides as bioreductive drugs: DACA N-oxide, nitractrine N-oxide and AQ4N", Br J Cancer, Suppl (1996), 74(27), S43-S47 See page 5243 columns 1-2	1, 15